TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

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# CONTROLLED DRUG DELIVERY FROM POROUS LACTIDE-BASED POLYMERS

Master's thesis

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and D. Sc. Niina Ahola
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Lääkkeenluovutuslaitteet ovat pieniä implantoitavia laitteita, jotka luovuttavat tasaisesti lääkeainetta ennalta määrätyn ajan verran. Lääkkeenluovutuslaitteisiin liittyy lukuisia hyötyjä verrattuna perinteisiin tapoihin, joihin kuuluu lääkeaineen ottaminen annoksena. Perinteisiin tapoihin, kuten suun kautta otettaviin pillereihin liittyy puolestaan useita haittapuolia. Lääkkeenluovutuslaitteilla pystytään lisäämään turvallisuutta ja tehokkuutta samalla kun potilaan vaste terapeuttiseen aineeseen paranee. Lisäksi sivuvaikutukset pystytään minimoimaan, kun laite on sijoitettu sille spesifiin paikkaan kehossa. Markkinoilla on ollut jo jonkin aikaa implantoitavia lääkettä vapauttavia laitteita, mutta ne ovat olleet suurimmaksi osaksi biohajoamattomia, jolloin kirurginen toimenpide tarvitaan niiden poistamiseksi. Biohajoaville implanteille, jotka poistuvat elimistöstä luonnollisia aineenvaihdunnan reittejä, olisi selkeäsi kysyntää.

Lääkkeenluovutuslaitteita on olemassa useita erilaisia, mutta tässä työssä keskityttiin matrix-tyyppisiin laitteisiin, joissa lääkeaine on tasaisesti jakautuneena biohajoavaan polymeerimatriisiin. Kokeellista osuutta varten valittiin kaksi erilaista lääkeainetta: askorbiinihapon suola ja deksametasoni. Polymeerit polymeroitiin laktidista ja kaprolaktonista etyleeni glykolin (PEG) ollessa ko-initiaattorina. PEG jää polymeroinnissa ketjun keskelle. Kaupallista kaprolaktonin ja L-laktidin P(CL-LA) polymeeriä käytettiin vertailukohtana. Lisäksi koesarjat tehtiin huokoiselle ja huokoistamattomille näytteille.

Karakterisointiin käytettiin menetelminä, differentaalista pyyhkäisykalorimetriä, termogravimetrista analyysia, geelipermeaatiokromatokrafia, kapillaariviskometriä sekä mikro-tietokonetomografia. Lääkeaineiden vapautumista seurattiin UV/VIS-spektrometrillä.

Lääkkeen vapautumiseen huomattiin vaikuttavan moni eri tekijä. PEG:n lisääminen polymeeriketjun keskelle lisäsi yleisesti lääkeaineen vapautumista. Valittu laktidin tyyppi vaikutti myös vapautumiseen. Lääkeaineen konsentaariolla ei havaittu olevan suurta vaikutusta vapautumisen profiiliin, mutta kinetiikkaan pystytään vaikuttamaan. Ylikriittisellä hiilidioksidilla prosessointi lisäsi yleisesti lääkeaineen vapautumista. Itse lääkeaine kuitenkin oli hyvin suuri tekijä lääkkeen vapautumisen kannalta. Askorbiinihapon johdannaisella oli heikko vuorovaikutus kaikkiin matriisipolymeereihin. Vapautuminen oli suhteellisen nopeaa useimmissa tapauksissa. Deksametasonin tapauksessa vapautuminen oli hyvin lähellä nollannen kertaluvun vapautumista. Näillä materiaaleilla on selvästi potentiaalia lääkkeenluovutussovelluksiin. Enemmän karakterisointia sekä materiaalin hajoamiskoesarja olisi suositeltavaa tehdä, jotta lääkkeenvapautumisen käyttäytymistä voisi ymmärtää paremmin.

# ABSTRACT

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Drug delivery devices (DDDs) are small implantable devices making sustained release of drug possible over defined time period. DDDs have numerous advantages compared to conventional ways of dosing drugs. Traditional ways like oral pills have numerous disadvantages. More safety and efficacy methods are needed while better patient compliance is achieved. At the same time, unwanted side effects can be minimized while drug is targeted into specific site with minimal released concentration. So far, mostly nondegradable DDDs have been available. Their major drawback is that they need removal after the drug is released. There is a need for biodegradable implants that are metabolised from body after DDD has performed needed actions.

There are many kinds of DDDs, but this thesis concentrated in matrix type biodegradable DDDs, where drug is homogenously dispersed in a matrix polymer. For study, two very different drugs were chosen: ascorbic acid salt and dexamethasone. Polymers were prepared by polymerizing lactide and caprolactone in presence of ethylene glycol as co-initiator. Block structure was formed where PEG was left in middle of polymer chain. Commercial copolymer of caprolactone and lactide, P(CL-LA) was used as comparative polymer. Drug release test series was done to both, porous and nonporous samples. Characterization was done by using techniques like differential scanning calorimetry, thermogravimetric analysis, size-exclusion chromatography, capillaryviscometry and microcomputed tomography. Drug release was monitored using ultraviolet/visible-spectrophotometer.

Many different factors were observed to have an effect on the drug release. In general PEG incorporation into backbone increased release rates. Also, type of lactide had effect to on the release. Content of drugs was not observed to have much effect on the release profile in general, but it was possible to tailor release rates. Processing samples with super critical $CO_2$ increased release rates of all samples. Most of all, properties of drugs affected in great extent to release kinetics and release profiles of drug-polymer combinations. AAs had relatively weak interaction with matrix polymers. Release was very fast in most of cases and standard deviations were relatively high in every measurement. For dexamethasone, sustained nearly zero-order kinetics was possible to achieve for some materials.

These materials clearly have great potential in drug release applications in future. More material characterization and degradation study could be useful to do for better understanding of behavior of used drug-polymer combinations.

# PREFACE

This master thesis was done in the Department of Electronics in Tampere University of technology. This work is related to project called Human spare parts funded by TEKES (Finnish Funding Agency for Innovation) but it is also related to project KURKO which is a commercializing project developing composite materials for bone applications.

I would like to thank all colleagues in Department of Electronics and Communications engineering for making the working environment such pleasant place to work. Special thanks I would like to give to laboratory staff: Heikki Liejumäki and Suvi Heinämäki for helping at laboratory. I also like to thank Sanja Asikainen, Kaarlo Paakinaho and Markus Hannula for all the help with I got with sample preparation, testing and analyzing. I would like to thank my supervisors, Niina Ahola and Minna Kellomäki, from all guidance and help for trouble shooting, I got during my thesis.

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Tampere

Anna-Kaisa Kyhkynen

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# ABBREVIATIONS AND EXPLANATIONS

| | |
|---|---|
| AAs | Ascorbic acid salt |
| Biodegradation | Loss of molecular weight |
| Bioerosion | Mass loss of polymer |
| DEX | Dexamethasone |
| i.v. | Inherent viscosity |
| microCT | Microcomputed tomography |
| $M_n$ | Number average molecular mass |
| $M_w$ | Weight average molecular mass |
| $sCO_2$ | supercritical carbondioxide |
| SEC | Size exclusion-cromatography |
| $T_g$ | Glass transition temperature |
| $T_m$ | Melting temperature |
| PCL | poly(ε-caprolactone) |
| P(CL-LA) | Poly(caprolactone-co-lactide) |
| PEG | Poly(ethylene glycol) |
| PEG-b-P(CL-LA) | Poly(ethylene glycol)-block-poly(caprolactone-co-lactide) |
| PLA | Poly(lactide) |

# THEORY PART

# 1 INTRODUCTION

Purpose of the work was to study drug release behavior of lactide-based porous biodegradable materials and characterization of them using different methods.
The theory part briefly introduces the principles of controlled drug delivery, possible mechanisms of release from biodegradable materials, factors affecting the release kinetics and degradation and drug release from porous materials. Used materials and drugs, are also introduced briefly.

In the experimental part, methods for material characterization were differential scanning calorimetry, thermogravimetric analysis, capillary viscometric analysis, size-exclusion chromatography and microcomputed tomography. Main interest was in drug release part, which was monitored using UV/VIS-spectrophotometer. Also initial drug contents were measured.

Theory and real drug release behavior from biodegradable polymers are very complex due to changes in material caused by constant changes in material. Aim was to recognize factors that affect the drug release profile and kinetics, and can be used to tailor properties of potential drug delivery devices.

In the literature, there are some drug release studies related to similar polymers that are used here. However, these are mostly dealing micro- or nanoparticles where the PEG block is much smaller than what we have used in this work. These studies are also for shorter time periods. No publications of similar porous materials were found. Additionally, $CO_2$ processing is relatively novel technique to prepare drug delivery devices.

# 2 CONTROLLED DRUG RELEASE FROM BIODEGRADABLE POLYMERS

## 2.1 Principles of controlled drug release

Controlled drug delivery means that active agent is combined with system which releases drug in a controlled way (Saltzman 2001; Bader & Putnam 2014). These are called as drug delivery devices (DDDs). DDDs are implanted to a specific site where implant releases active agent over extended period of time. Local drug concentration is kept at desired level while unwanted side effects are minimized. (Bader & Putnam 2014).

Conventionally drug is taken in oral doses. One dose is effective only short period of time. (Jones 2004) *Figure 1* presents how drug concentration varies between doses. Dotted line presents pulsatile dosage and solid line controlled release dosage. Plasma concentration should be kept inside the therapeutic window. It means that concentration is kept between maximum safe concentration and minimum effective concentration. (Bader & Putnam 2014) Orally taken drugs have many issues like poor patient compliance. (Jones 2004)

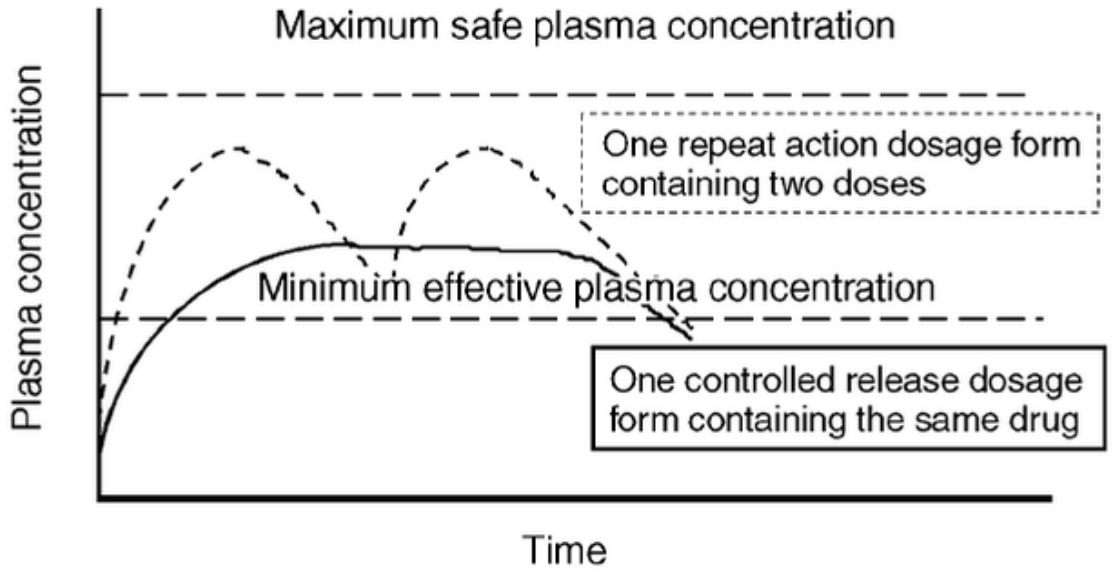



***Figure 1.*** *Schematic presentation of typical drug concentration as function of time. (Jones 2004)*

Different kinds of non-degradable drug delivery systems have been available for a while now. There is strong motivation to develop biodegradable DDDs. Conventional methods (for example oral pills) in drug delivery have different kinds of issues like unwanted

side effects and possible toxicity of drug. Newer methods can possibly solve many of them. Safety and efficacy of drugs can be improved and proteins and other difficult drugs need something else than conventional methods to be delivered for example. (Langer 1990) Biodegradable DDDs do not need removal surgery like non-degradable ones which is a great advantage. (Bader & Putnam 2014) Disadvantages of biodegradable DDDs are that these may be very complex and costs of development can be expensive. (Kleiner et al. 2014)

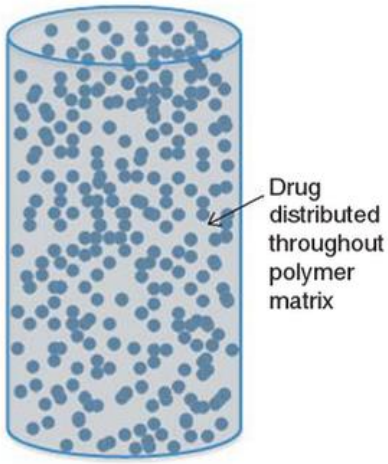



***Figure 2****. Schematic presentation of matrix device. Modified from (Bader & Putnam 2014).*

There are several different kinds of drug delivery devices but in this work focus is in biodegradable matrix devices (*Figure 2*). Matrix device means that drug is homogenously dispersed in a polymeric material. (Jones 2004)

## 2.2 Drug release mechanisms from biodegradable polymers

### 2.2.1 Diffusion

Diffusion can be described as random movement of substances (drug) from high concentration region to low concentration region (Jones 2004). There are several different factors that have an effect on diffusion. Physical properties have important role (Willerth & Sakiyama-Elbert 2007). Kinetics of release can be determined by concentration gradient, diffusivity of substance inside polymer matrix and mean diffusion distance. (Szentivanyi et al. 2011) Fick's First Law can be used to model simple one direction diffusion flux, J (mass flow/area):

$$J = -D\frac{\partial c}{\partial x}; \tag{1}$$

where D is the diffusion coefficient, c solute concentration and x distance. (Siegel & Rathbone 2012) It is used when diffusion is expected to be steady state (Lao et al. 2011).

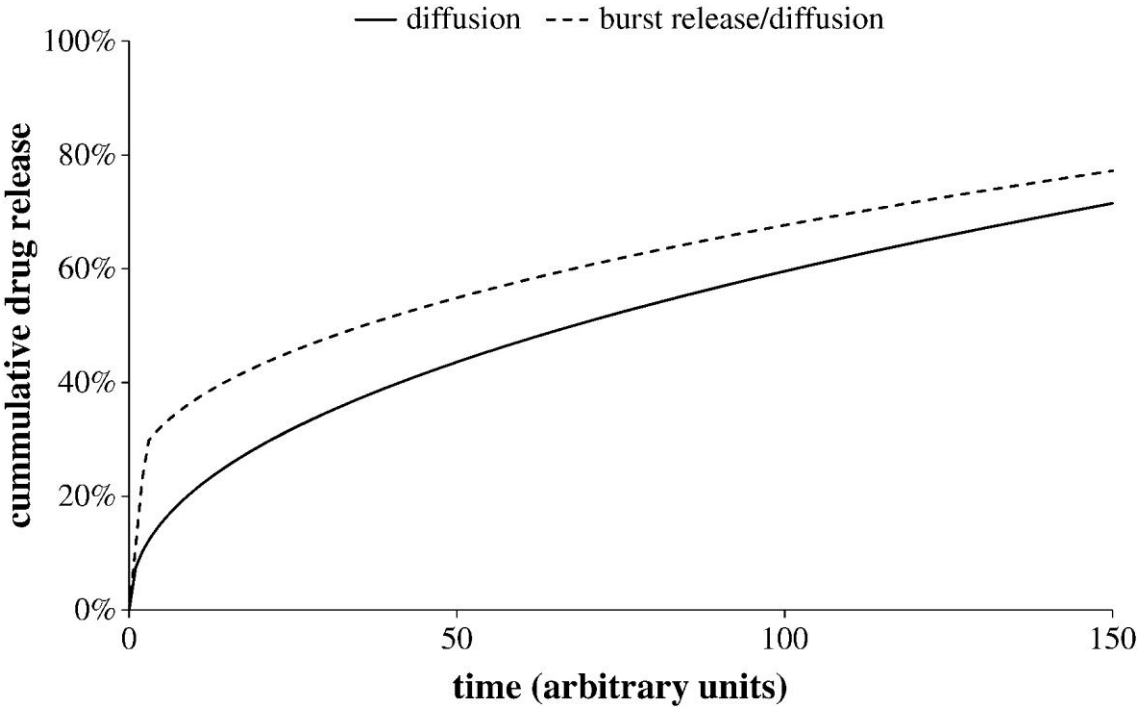



***Figure 3**. Typical release profile based on diffusion. (Szentivanyi et al. 2011)*

*Figure 3* presents typical diffusion based drug release profile. In diffusion based devices, the rate of drug release decreases with time because distance of diffusion increases (Siegel & Rathbone 2012).

### 2.2.2 Bioerosion

When release is controlled by erosion, diffusion of substance is negligible inside a polymer. Drug is released by degradation and erosion of matrix. (Szentivanyi et al. 2011) Polymer erosion is defined as decrease in mass and degradation as decrease in molecular weight (Bader & Putnam 2014; Szentivanyi et al. 2011; Lao et al. 2011). The rate of degradation is dependent on the availability of water molecules and how sensitive the chemical bonds of the polymer backbone (Szentivanyi et al. 2011). Material can be bulk or surface degradable. Difference between these two is illustrated in *Figure 4*.

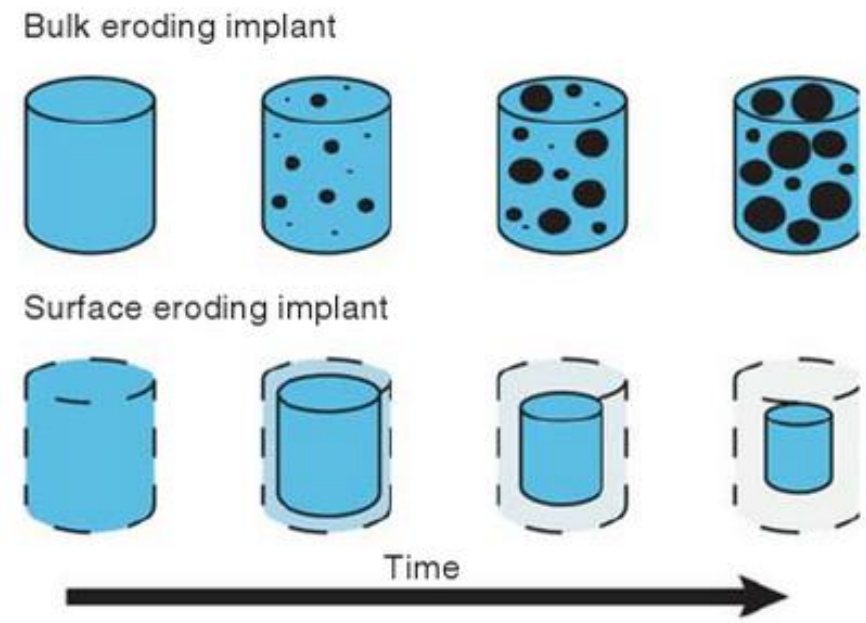



***Figure 4**. Schematic presentation of bulk and surface eroding implants. (Bader & Putnam 2014)*

If polymer degradation is faster than water diffusion into polymer, polymer is surface erodible and vice versa because degradation is dependent on presence of water molecules. (Bader & Putnam 2014). With surface erodible materials, the drug release often correlates well with mass loss of polymer. These are usually hydrophobic polymers. (Ratner et al. 2013) Polymer hydrophobicity/hydrophilicity has an important role on how polymer degrades (Szentivanyi et al. 2011; Bader & Putnam 2014). With surface eroding polymers, near zero order release is possible to achieve (Siegel & Rathbone 2012).

With bulk erodible polymers, diffusion of drug has important role (Ratner et al. 2013). In *Figure 5* different stages of drug release from erodible polymer are presented. For bulk eroding polymers typically burst effect is observed (Rich et al. 2002). This is because first the drug releases from surface and from pores near the surface (**a** in Figure). Usually, the aim is to finish the drug release before degradation starts (Rich et al. 2002). Next stage is latent stage (**b**). Some degradation is seen, but some of the drug is trapped. Finally (**c**) rest of drug is released rapidly because of autocatalytic degradation. Polymers that degrades by bulk should not be used with drugs that have narrow therapeutic window (Siegel & Rathbone 2012). However, usually both, surface and bulk erosion occurs at same time with polymers.

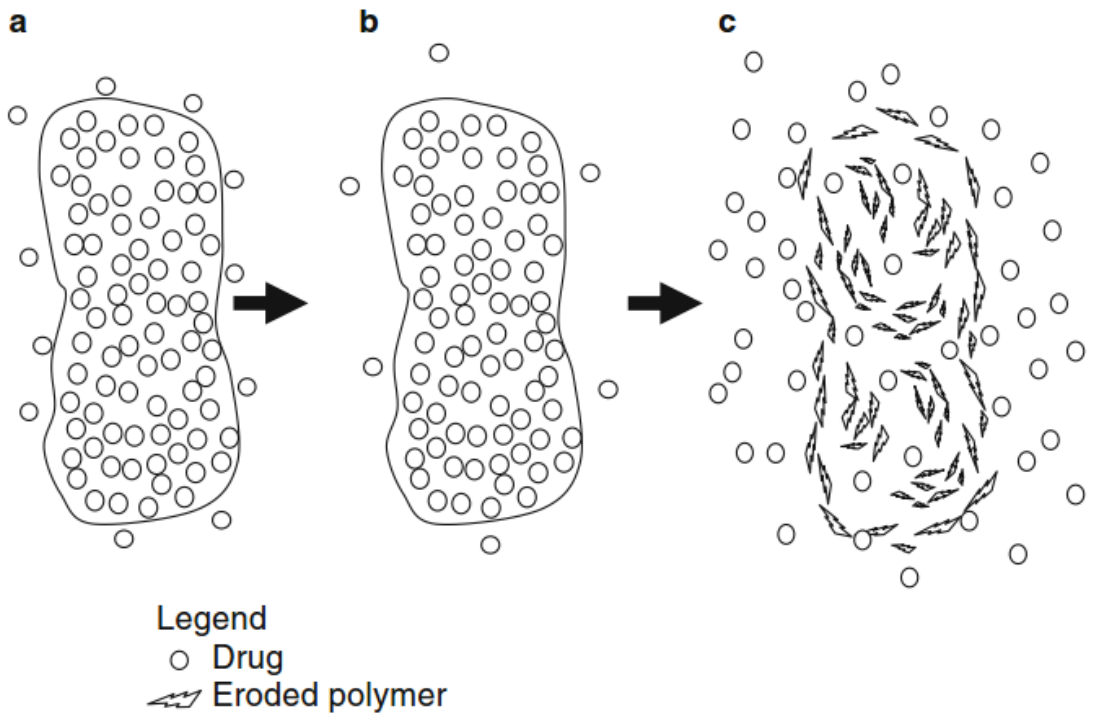



***Figure 5**. Schematic presentation of different stages of bulk eroding and drug releasing polymer. (Siegel & Rathbone 2012)*

There are three different ways for bioerosion. First group is water solubilized polymers that have been insolubilized. Solubility of the drug has to be taken into account because the drug is in aqueous environment. Well soluble drugs are released rapidly. Second group is water insoluble polymers that are solubilized by hydrolysis, ionization or protonation of a pendant group. Finally, the third group is hydrophobic polymers that are converted into water-soluble molecules by backbone cleavage. (Heller 1979)

### 2.2.3 Chemical bond

Drug can be covalently or non-covalently bonded to a polymer (Willerth & Sakiyama-Elbert 2007). This allows protein and growth factor delivery in a way that active agent will not lose its activity (Pasut & Veronese 2007). However in design it has to be taken into account that drug will not lose its biological activity because of chemical reactions. (Willerth & Sakiyama-Elbert 2007). Usually drug is activated biologically when bond between drug and polymer is cleaved because of hydrolysis (Baker 1987). Hydrolysis is rate limiting factor in chemically releasing materials. (Baker 1987) *Figure 6* illustrates different types of approaches to synthesize chemically controlled drug delivery device.

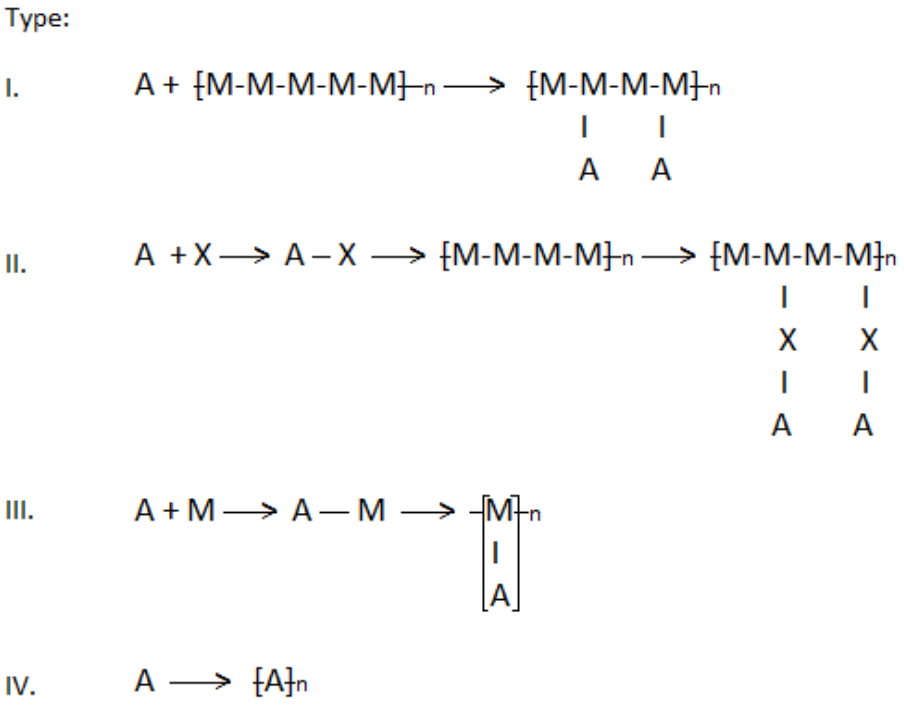



***Figure 6****. Different approaches to synthesize chemically bonded drug-polymer system. A is active agent, M is polymer and X is labile group. Modified from (Baker 1987)*

These can be classified into different groups: Drug (Type I) has already reactive group that is bonded to polymer backbone. If it does not contain reactive group, drug molecule can be manipulated to be reactive like in type II. Drug can be converted into derivative that can be polymerized (Type III) or vary rare situation (Type IV) where drug can be directly converted into polymer. (Baker 1987)

## 2.3 Release kinetics

In reality, erosion and diffusion occur at the same time which makes predicting of release kinetics difficult (Lee et al. 2003). There are numerous factors that have an effect on the process. For polymer degradation and drug release, at least crystallinity of polymer, drug molecular size and solubility and morphology are factors that affect to these processes. (Lee et al. 2003) Crystallinity makes the material more close packaged which leads to decrease of diffusion. Thus, crystallinity is an important factor in drug release. Polymer backbone composition has an important role in controlling the rate of erosion. (Bader & Putnam 2014)

Degradation of material is quite difficult to predict due to the physical changes in material during degradation. Even though degradation process is complex, release kinetics of drugs can be similar with non-degradable ones. (Saltzman 2001) Also diffusivity of degrading material changes as function of time (Bader & Putnam 2014). *Figure 7*

presents how it affects the release profile. Black line presents degradable implant and gray line nondegradable implant.

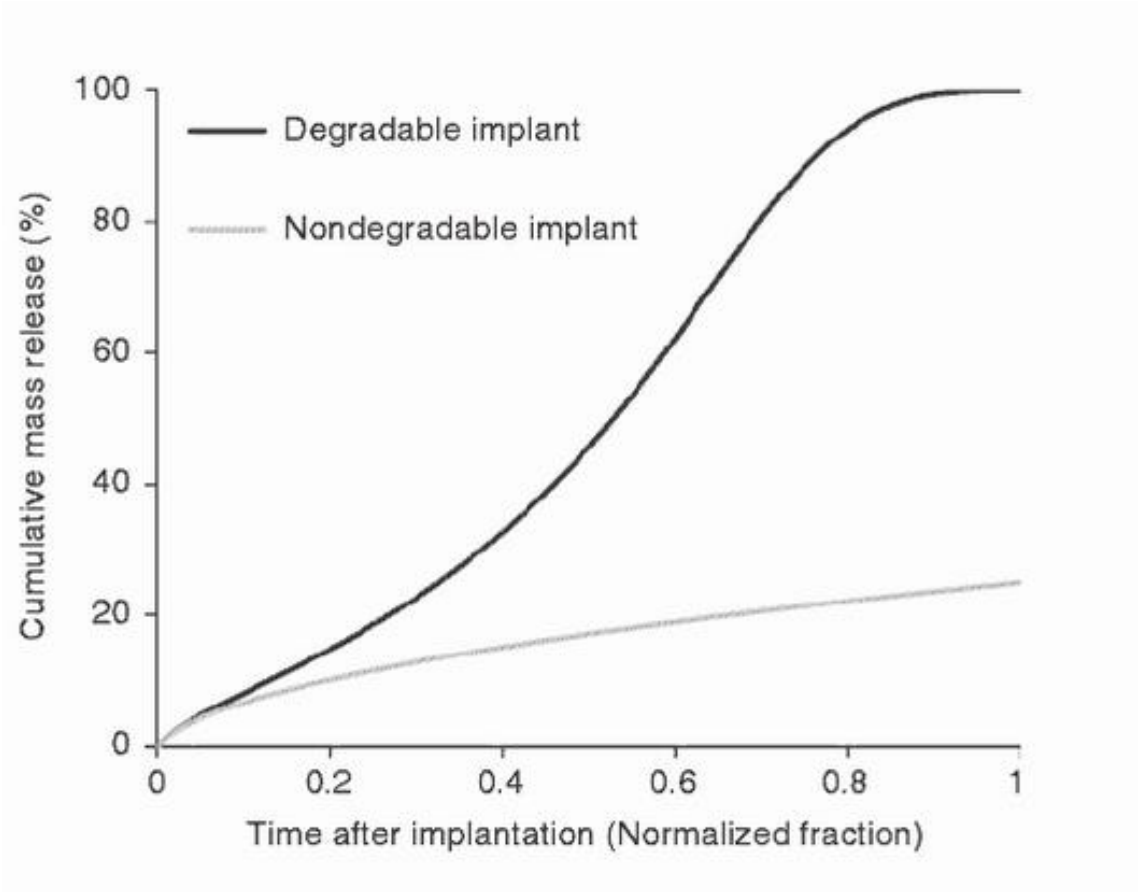



***Figure 7*** *Difference between release profile of degradable and non-degradable bulk polymers. (Bader & Putnam 2014)*

*Figure 8* presents typical release profiles based on diffusion, diffusion and bulk erosion and surface erosion.

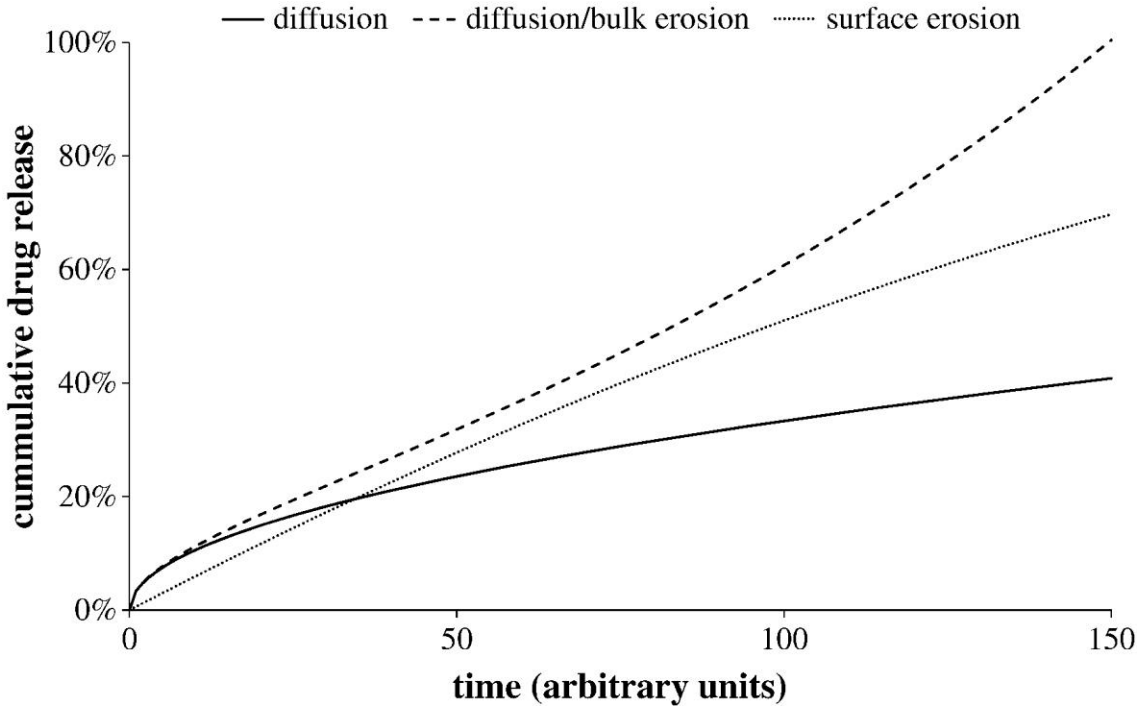



***Figure 8****. Schematic presentation of different release profiles in different kinds of release mechanics.(Szentivanyi et al. 2011)*

Chemical properties of the polymer and the drug have great effect on the release. Drug distribution, molecular weight of polymer and polymer blending are also factors worth of mention. (Freiberg & Zhu 2004) Drug can be dispersed or dissolved in the polymer (Saltzman 2001). Usually release of hydrophobic drugs from hydrophobic material is very slow (Zilberman et al. 2010) and on the contrary well watersoluble drugs may release very rapidly as mentioned before. Lipophilic drugs often get trapped in the hydrophobic polymer resulting very slow release (Tamboli et al. 2013). Burst effect can be possibly reduced by forcing drug to dissolve/disperse better into polymer matrix by using surfactants or cosolvents. Also drug loading amount and porosity of material are important factors for burst effect. These are studies where has observed to have relation between these factors and burst effect. (Rothstein & Little 2011)

Sample size and shape have an effect on the degradation rate (Yoon et al. 2003). When implants have defined geometry, it is possible to predict and simulate release (Bader & Putnam 2014). There are numerous different models available in literature. Probably the most known and successful model for matrix release system is Higuchi model. It is also based on Fick's first law (Lao et al. 2011). However, it is for systems that do not go through erosion. Erosion causes changes in matrix by increasing permeability of drug. (Heller 1979)

With polyesters, it is known that the acidic degradation products catalyze the degradation process (Szentivanyi et al. 2011). Size and shape of matrix has effect how sensitive material is to catalysis (Lao et al. 2011). *Figure 9* presents process of acid and alkaline based hydrolysis. Carboxylic acid and alcohol are formed in the process.

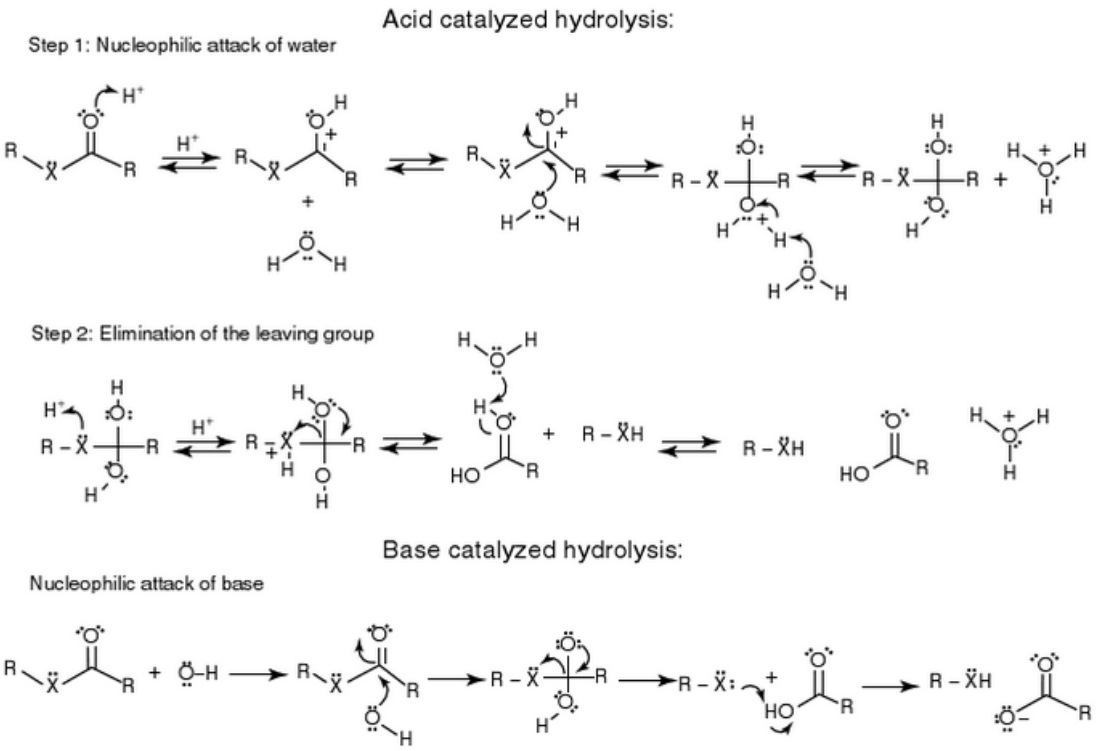



***Figure 9****. Hydrolysis of ester in an acidic and alkaline environment (Bader & Putnam 2014).*

When hydrolysis is acid catalyzed, the reaction has two stages. Free hydrogen associates with carbonyl while water acts as nucleophile. Tetrahedral intermediate is formed which makes alcohol to leave easily. In alkaline catalyzed environment, free hydroxyl anions acts as nucleophile. Again this nucleophile causes formation of tetrahedral intermediate which leading to alcohol elimination. Reaction goes on until polymer has degraded completely. (Bader & Putnam 2014)

## 2.4 Degradation and drug release of porous materials

It is known that porosity of material affects the degradation of material. However effect of pore size to degradation process is not well known yet. There are somewhat conflicting results available. (Odelius et al. 2011)

Degradation can occur faster with nonporous specimens because the products of degradation have easier path to the surrounding solution (Odelius et al. 2011). Especially, with polyesters' acidic degradation products tend to cause an auto catalytic effect when acidic degradation products are trapped inside polymer matrix (Dash & Konkimalla 2012). Odelius et al. (2011) studied degradation of solid and porous PDLLA (L/D 96/4) films. Solid films and large pore sized samples degraded fastest. It was suggested that autocatalytic effect took place. Degradation products are thought to get trapped inside material. Smaller pore size samples degraded slower than the other samples. (Odelius et al. 2011)

Lu et al. (2000) studied degradation behavior of porous (70-90 -wt%) PLLA foams. Again, autocatalytic effect was thought to take place in in vitro degradation test series. They concluded that increase in pore wall thickness caused weight average molecular weight to decrease significantly due to autocatalysis. Degradation products can be trapped inside polymer matrix. (Lu et al. 2000)

Pore structure also has an effect on the drug release kinetics (Siegel & Rathbone 2012). Velasco et al. (2010) studied degradation of polymer and release of ibuprofen from porous Poly(methyl methacrylate)-Poly(lactide) blends processed using supercritical $CO_2$. They concluded that swelling and degradation behavior were dependent on porosity and PLA content of samples. Release was faster from samples with higher swelling and degradation.

Whang et al. (2000) studied protein release from highly porous PLGA scaffolds prepared using emulsion freeze-drying technique, having general porosity approximately 90%. Protein was added 0.1 mg/ml or 0.2 mg/ml in emulsion, and poresize was varied between 7-70μm. It was concluded that smaller pore sized samples showed slower release rate than bigger pore sized samples having same amount of drug.

Yoon et al. (2003) studied dexamethasone release from porous PLGA scaffolds. Release rate was dependent on the initial drug content. No burst effect was seen at beginning of test series and release was controlled over 30 days. Released drug was able to suppress proliferation of lymphocytes and smooth muscle cells in in vitro.

Martin et al. (2001) used porous polymer foams made of PLGA and PEG. Polymer were conjuncted with different molecules to differentiate bone marrow stromal cells into cartilaginous of bone-like tissues.

# 3 MATERIALS IN CONTROLLED DRUG RELEASE

## 3.1 Synthetic biodegradable polymers in drug delivery

### 3.1.1 Polylactide

Polylactide is an aliphatic polyester having repeating unit of lactic acid. Aliphatic polyesters are most studied polymers in therapeutic field (Kleiner et al. 2014; Bastioli 2005). There are two isomers that can be used. These are named as L- and D-lactic acid (Jones 2004). These are presented in Figure 10.

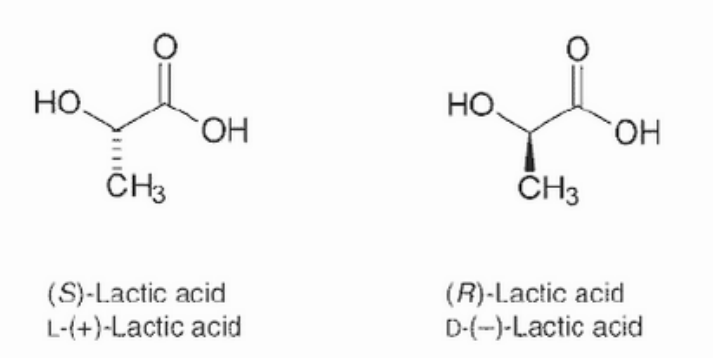



***Figure 10**. Isomers of lactic acid.* (Auras et al. 2010)

PLA is polymerized using ring opening polymerization of lactide, a dimer of lactid acid (Bastioli 2005). Structure of poly(lactide) is presented in *Figure 11*.

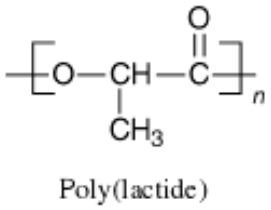



***Figure 11**. Structure of Poly(lactide). (Jones 2004)*

Poly-L-lactide (PLLA) is a semi-crystalline polymer with melting temperature ($T_{m)}$ of 175-180 °C and glass transition ($T_{g)}$ of 60 °C. It is brittle by nature and decomposes around 185 °C. L-lactic acid is usually copolymerized with D-lactic acid or other hydroxyacids to obtain better processing characteristics and lower $T_g$. (Bastioli 2005) Degradation takes about 18-24 months (Saltzman 2001). On the contrary to PLLA, poly-D,L-lactide (PDLLA) is amorphous and degrades in weeks (Bramfeldt et al. 2007).

Paakinaho et al (2009) studied in vitro degradation of PDLLA (96/4) with different molecular weights. It was concluded that rheological parameters affected also to degradation of material.

PLA degrades into lactic acid by hydrolysis of ester bonds. Degradation products are removed from body by normal metabolic ways. (Lu et al. 2000) PLA hydrolysis can be autocatalyzed by acidic degradation products (Bastioli 2005). In drug delivery it is known to be less permeable than PCL (Pitt et al. 1979).

### 3.1.2 Poly(ε-caprolactone)

Poly(ε-caprolactone) (PCL) is a linear thermoplastic biodegradable polyester (CRC n.d.). It is semi-crystalline and has relatively polar ester group and five non-polar methylene groups (Wei et al. 2009; Tamboli et al. 2013). Structure of PCL is presented in *Figure 12*. It has low $T_g$ around -60 C (Bastioli 2005; Bramfeldt et al. 2007) and $T_m$ around 59-64 C (Saltzman 2001). PCL is more flexible and more hydrophobic than PLA. (Bastioli 2005) It is known from very good biocompability (Dash & Konkimalla 2012) and from good permeation to drugs (Bramfeldt et al. 2007). Its hydrophobic nature makes encapsulation efficiency of lipophilic drugs good (Tamboli et al. 2013). It also has excellent miscibility with many polymers (Hiljanen-Vainio et al. 1996).

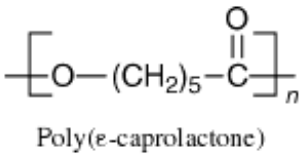



***Figure 12****. Structure of PCL. (Jones 2004)*

PCL is synthetized by ring opening polymerization of ε -caprolactone (Wei et al. 2009). Degradation takes approximately 30 months depending on conditions of environment (Saltzman 2001). Degradation starts from amorphous regions and it is autocalatyzed by carbonyl end group that fragments from matrix. Water permeability into material is rate limiting factor in degradation process. It takes from 4 to 6 months for start of mass loss. (Dash & Konkimalla 2012) It degrades slower than PLA, which makes it suitable for longterm applications (Saltzman 2001). However, copolymerization leads often to faster degradation (Saltzman 2001). Physical, chemical and mechanical properties can be tailored by copolymerizing or blending with other polymers. Copolymerization is often done with other hydrophilic monomers. (Dash & Konkimalla 2012)

### 3.1.3 Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) is also known as poly(ethylene oxide) (PEO). Structure of PEG is presented in *Figure 13*. It is synthetized from ethylene oxide by ring opening (Pfister & Morbidelli 2014).

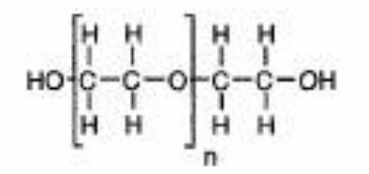


***Figure 13****. Structure of poly ethylene glycol. (Jones 2004)*

It is hydrophilic polymer having high solubility in water but also in various organic solvents (Saltzman 2001; Pfister & Morbidelli 2014; Bramfeldt et al. 2007). Water molecules bind to PEG structure (Pfister & Morbidelli 2014). PEG has excellent biocompatibility. It does not go through hydrolysis but incorporation into copolymer backbone has shown to have role in degradation process. Incorporation of PEG into polymer backbone has shown to have increasing effect on degradation. It makes material more hydrophilic and water uptake higher. (Bramfeldt et al. 2007)

PEG gives opportunity for many different kinds of drug release systems. There are numerous studies of different protein delivery systems (Veronese & Pasut 2005) In general, it increases the solubility of drugs (Zhang & Zhuo 2005) Hydroxyl groups of PEG allow copolymerization with lactides, glycolides and caprolactone for example (Li et al. 1998).

Excretion from body can be an issue. Normally, it is excreted in urine or feces but high molecular weight PEG may accumulate to liver, which may lead to macromolecular syndrome. (Veronese & Pasut 2005) However molecular weight below 20 000g/mol filtrates though kidneys. (Li et al. 1998)

### 3.1.4 Poly(L-lactide-co-caprolactone)

Structure of Poly(L-lactide-co-caprolactone), P(LA-co-CL) is presented in *Figure 14*.

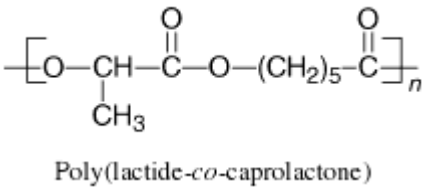



***Figure 14****. Structure of P(LA-co-CL). (Saltzman 2001)*

Ahola et al. (2013) Studied hydrolytic degradation of Poly(L-lactide-co-caprolactone) with the comonomer ratio of 70/30 with different β-TCP contents (0-50%). TCP did not

have effect on the degradation of the matrix. Composites absorbed water more than plain polymer. For all samples, the mass loss was very small during the first ten weeks. Water absorption of plain polymer increased rapidly after 20 week time point. $T_g$s of samples decreased until 12th week timepoint was reached. It was same time point when molecular weights started to decrease rapidly. Melting temperatures increased from 2nd week time point to 16$^{th}$ week time point from 111-113°C to 116-120°C. After 16$^{th}$ week melting points decreased constantly. (Ahola et al. 2013)

Ahola et al. (2012) Studied hydrolytic degradation and in vitro rifampicin release from composites of Poly(L-lactide-co-caprolactone) 70/30 and β-TCP. Degradation of materials obeyed first order kinetics. Decrease of molecular weight was relatively rapid. Composites including rifampicin degraded more rapidly at beginning of test series than samples without TCP. Mass loss and water absorption started earlier than in study of ciprofloxacin release (Ahola et al. 2013). It was suggested that Rifampicin's more hydrophilic nature caused this kind of behavior. Four different phases were found during release. Samples without ceramic fillers had quite long lag phase at start. (Ahola et al. 2012)

In drug release applications P(DLLA-CL) with block structure is known from burst effect and poor water absorption after amorphous lactide units has degraded rapidly. It is not kind of behavior that is needed in drug release applications. However, more randomized structure may degrade in more stable way and show more controlled drug release behavior. Additionally, by varying ratio of LA/CL unit, it is possible to control the degradation of polymer. (Bramfeldt et al. 2007)

Pitt et al. (1979) studied steroid release from P(DLLA-CL) with five different drugs and varying LA/CL ratio. PDLLA were 1000 times less permeable than PCL. Since PDLLA is totally amorphous the poor permeability was thought to be cause from decrease of free volume. However it was significantly increased by using additives. Copolymers of D,L-lactide and caprolactone had good permeabilities. (Pitt et al. 1979)

in at study of Hiljanen-Vainio et al. (1996) degradation of copolymers of caprolactone and lactide were studied. Ratio of LA/CL and type of lactide varied. Properties of polymers varied from very elastic materials to tough material. Mechanical values such as tensile modulus and tensile stress were higher with every homopolymer compared to copolymers but maximum strains were relatively low. Malin et al (1996) continued degradation study of copolymers of caprolactone and lactide. Also pure PLLA, PDLLA and PCL were studied as comparison. Molecular weights of copolymers decreased rapidly at beginning of hydrolysis. However, any significant mass loss was not seen. (Malin et al. 1996)

Water absorptions were for PLLA, PDLLA and PCL after 1 week 4.7, 20.4 and 0.5-wt% respectively. After two week timepoint, PDLLA absorbed 38.6-wt% water and was not measurable after that. During 7 week hydrolysis crystalline PLLA absorbed 18.3-wt% of water while PCL did only 0.1-wt%. (Malin et al. 1996) Karjalainen et al. (1996) continued research by studying changes in mechanical properties after in vitro of same materials that was used Malin et al (1996) in their study. Copolymers kept their me-

chanical properties like tensile modulus better than homopolymers of lactide. Homopolymer of caprolactone kept its properties almost at same during 70 days of hydrolysis at 23 °C. (Karjalainen et al. 1996)

Copolymers of ε-CL and D,L-LA were also studied by Hiljanen-Vainio et al. (1997). Content of ε-CL was varied between 5 to 30-wt%. Again, dramatic weight loss was seen by following mass loss weeks later. Tensile tests were performed to materials. Mechanical properties varied from hard and brittle to rubbery like material. ε-CL brings elasticity to material.

Monomer content has very important role for properties of material. Having 85-wt% of DL-lactide and 15-wt% ε-CL makes material rubberylike, but increasing DL-LA content to 90-wt% changes properties to rigid. (Hiljanen-Vainio et al. 1997)

### 3.1.5 Poly(lactide-co-caprolactone)-poly(ethylene glycol)

Lactides, glycolides and caprolactone give numerous opportunities to create interesting materials. Properties can be tailored with varying different factors like for example lactide/caprocatone ratio and type of lactide monomer. It is not surprise that there are also studies related to different combinations available.

For example Bramfelt et al. studied P(CL-co-DLLA)-PEG-P(CL-co-DLLA) copolymers and effect of CL/DLLA ratio to degradation and material properties. They noticed that PEG was able to crystallize in this kind of material. Additionally, it was noticed that presence of D,L-LA had reducing effect to PCL crystallinity. It was clear that higher LA-content was consistent with higher water absorption and increasing mass loss. PEG had role of increasing hydrophilicity. (Bramfeldt et al. 2007)

Cho et al. studied effect of PCL/PDLLA unit composition to degradation of P(D,L-LA-ran-CL)-b-PEG-b-P(D,L-LA-ran-CL) films, where lactide and caprolactone have random structure with PEG block in the middle of polymer chain. $M_w$ of PEG was 200 g/mol while D,L-LA/CL ratio varied. Water absorption and mass loss were greater when D,L-LA/CL ratio was increased. It was explained by reduced crystallinity. (Cho & An 2006) Water absorption rates were less in this study than in Bramfeldt's study. It was suggested that that was due to smaller PEG segments (Bramfeldt et al. 2007).

Li et al studied degradation of PLLA-PEG-PLLA block copolymers. $M_w$ of used PEG was 1800g/mol. Ratio of LLA/EG was varied and it was noticed that PEG chain length had significant effect to water absorption and mass loss. Polymers were prepared using $CaH_2$ or Zn as coinitiator in synthetization. Used coinitiator had effect to these properties. It was suggested that $CaH_2$ prepared polymers were more random than Zn which leads to more amorphous samples. (Li et al. 1998)

Tamboli et al. (2013) prepared (PLA-PCL-PEG-PCL-PLA) pentablock nanocopolymers to study release of hydrophobic molecules. Different ratios of PEG/PCL/PLA were studied. Also the effect of L- and D-forms of lactide was studied. Degradation was faster compared to pure PLA and PCL. Slow release of triamcinolone acetonide, a corticosteroid, was observed from polymers PLLA-PCL-PEG-PCL-PLLA

(1/2,5/2,5 ratio) and PDLLA-PCL-PEG-PCL-PDLLA (1/2,5/2,5 ratio) which crystallinity and hydrophobicity were low compared to other studied polymers. Release was continuous for 35 days and burst effect was also relatively small. It was suggested that incorporation of lactic acid into copolymer reduced burst. (Tamboli et al. 2013)

Karjalainen et al. (2000) studied drug release of theophylline and propranolol (including 2-30-wt%) from P(CL-DLLA) copolymers prepared using glycerol, PEG 1000 or PEG 4000 as initiators. Increase of hydrophilicity resulted in higher release rates with both model drugs. PEG incorporation into backbone increased water uptake and rate of degradation.

## 3.2 Model drugs in release study

### 3.2.1 Dexamethasone

Dexamethasone **(**(11β,16α)-9-Fluoro-11,17,21-trihydroxy-16-methylpregna-1,4-diene-3,20-dione) has a molecular formula of $C_{22}H_{29}FO_5$ and molecular weight of 392,46 g/mol. It has a melting point of 262 °C and solubility in water is only 0,09 g/1000g at 25 °C (Heynes 2014). Dexamethasone has hydrophobic nature (Yoon et al. 2003). Structure of dexamethasone is presented in *Figure 15*.

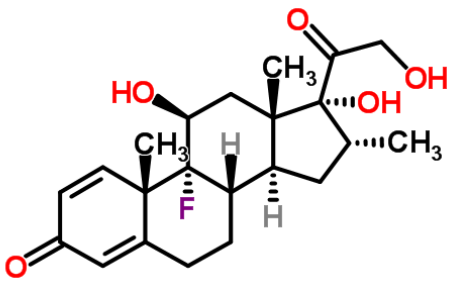



***Figure 15**. Structure of dexamethasone. (ChemSpinder n.d.)*

Dexamethasone is a glucocorticoid, synthetic steroid having anti-inflammatory effects (Willerth & Sakiyama-Elbert 2007; Yoon et al. 2003). It is commonly used to treat arthritis and sclerosis (Willerth & Sakiyama-Elbert 2007)

Dexamethasone inhibits smooth muscle cell proliferation and has an important role in regulation of cellular growth and division. It has been used to inhibit abnormal migration and proliferation of smooth muscle cells after restenosis. (Yoon et al. 2003)

Dexamethasone is traditionally used in osteoblast cell culturing (Wu et al. 2011). Martin et al. (2001) used dexamethasone with growth factor to guide bone marrow stem cells into osteoblast stem cells.

### 3.2.2 Vitamin C and its derivatives

Ascorbic acid or commonly vitamin C has molecular weight of 176,126 g/mol and solubility in water 246 g/1000g at 25 °C. (Yaws 2012)

Ascorbic acid is simple vitamin and was the first one to be isolated and purified. (Davies et al. 1991) Structure of ascorbic acid is presented in *Figure 16* and one of its salt derivatives, 2-Phospho-L-ascorbic acid trisodium salt, in *Figure 17*.

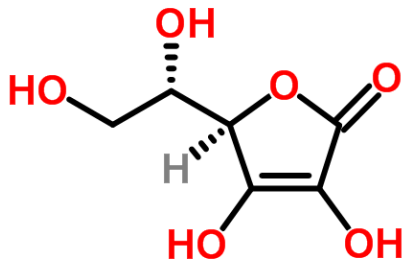



***Figure 16**. Structure of vitamin C. (ChemSpinder n.d.)*

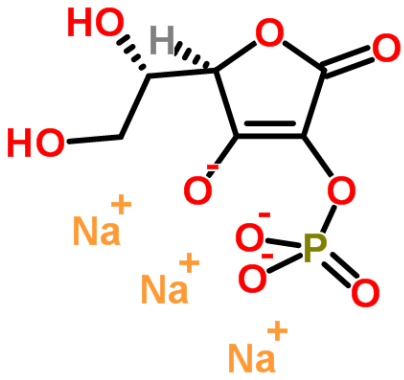



***Figure 17**. Structure of 2-Phospho-L-ascorbic acid trisodium salt. (ChemSpinder n.d.)*

It is the most industrially produced vitamin but also naturally found throughout in plant and animal kingdom. Its role is not very well understood in many of the processes it is involved. (Davies et al. 1991) It is commonly used in cosmetics and dermatological products. (Špiclin et al. 2003; Huang et al. 2013) It is an antioxidant and destroys oxidizing agents and free radicals that are involved in skin aging process but it is known to simulate collagen synthesis too (Špiclin et al. 2003).

However, it is very unstable so usually more stable derivatives are used (Špiclin et al. 2003)

It is known to easily oxidized by dioxygen ($O_2$). It is also commonly used in food industry. It can be used as an additive like for example improve taste and nutritional value, act as stabilizer or prevent oxidation in food (Davies et al. 1991)

In medical field, it has had many uses too. It is often used with other drugs. It has been used in osteogenic cell differientiation with dexamethasone and beta-glyserophosphate. (Wang et al. 2010)

# EXPERIMENTAL PART

# 4 MATERIALS AND METHODS

## 4.1 Materials

Medical grade Poly(L-lactide-co-ε-caprolactone) 70/30 was purchased from Corbion, Gorinchem Netherlands (code PLC 7015) to be used as comparable to experimental polymers. For polymerization of experimental polymers, ε-caprolactone (Fluka, Buchs, Switzerland) was used as distilled and D, L- lactide (Corbion, Gorinchem, Netherlands) was dried in vacuum and used as received. 0.05mol-% Sn(II)octoate (stannous 2-ethylhexanoate) (Sigma-Aldrich, Steinheim, Germany) was used as catalyzer and used as received. 0.035% co-initiator polyethylene glycol, dried in vacuum, was used as received.

Used drugs were 2-phospho-L-ascorbic acid trisodium salt having purity of 95% ($C_6H_6Na_3O_9P*xH_2O$, Lot: # BCBM4646V, Sigma, Germany) and Dexamethasone ($C_{22}H_{29}FO_5$, Lot # BCBK5387V, Sigma ) having purity of 98%. Drugs were used as received.

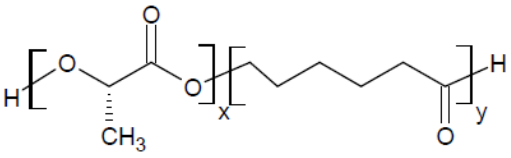
For Sörensen buffer solution, prepared using standard of ISO 15814 (Implants for surgery - copolymers and blends based in polylactide – in vitro degradation testing), potassium dihydrogen phosphate ($KH_2PO_4$) (J.T. Baker, Netherlands) and sodium phosphate dibasic anhydrous ($Na_2HPO_4$) (J.T. Baker, Netherlands) were used.

***Table 1***. *Polymers used in in vitro drug release study.*

| **Copolymer** | **CL-content (mol fraction)** | **LA-content (mol fraction)** | **Type of lactide** | **PEG in backbone** |
|---|---|---|---|---|
| **P(CL30/LLA70) (Corbion, Netherlands)** | 30 | 70 | L | - |
| **PEG-P(CL30-LLA70)** | 30 | 70 | L | yes |
| **PEG-P(CL30-DLLA70)** | 30 | 70 | DL | yes |
| **PEG-P(CL15-DLLA85)** | 15 | 85 | DL | yes |

Materials used for the in vitro drug release test series are listed in *Table 1*. First material is fully commercial and last tree was polymerized in Aalto university (Espoo, Finland) by Sanja Asikainen. Size of PEG block was 20 000 g/mol. Numbers following monomer abbreviations are mol fractions in feed. Structures of used copolymers are presented in *Figure 18*.

Poly(L-lactide-co-caprolactone)



Poly(lactide-co-caprolactone)-b-PEG-b-Poly(lactide-co-caprolactone)

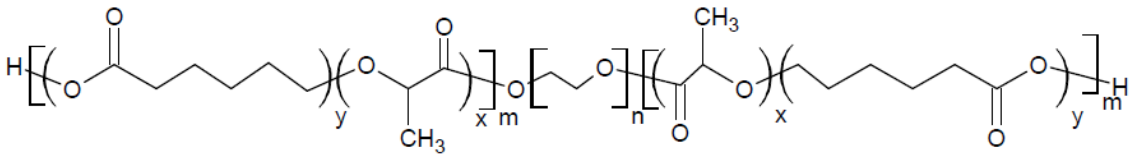


***Figure 18****. Chemical structures of used copolymers.*

A structures of used drugs were presented already in chapter 3.2. Dexamethasone (DEX) is in *Figure 15* and a derivative of ascorbic acid salt (AAs), 2-phospho-L-ascorbic acid trisodium salt, in *Figure 17*.

## 4.2 Methods

### 4.2.1 Polymerization and preparation of samples

Reactor was flushed with nitrogen for 15 minutes and 10 minutes when all reagents were inside. Reactor was closed and heated up to 160 °C. Polymerization times were 3hours 15minutes for PEG-P(CL30-LLA70), 4hours 30 minutes PEG-P(CL30-DLLA70) and 4hours 20 minutes for PEG-P(CL15-DLLA85).

Polymers were dissolved into dichloromethane and precipitated from ethanol and removed from liquid using tweezers. Polymers were left in fume chamber to dry over night and later in desiccator.

Before blending drugs with polymer, materials were dried in vacuum at least 24 hours. Polymer and drug was fed in turn into twin screw midi-extruder (DSM, capacity of 16 cm3 with screw length 150 mm) under nitrogen atmosphere. Blend was taken out once and feeded in again. After everything was inside, blend was used as a batch mixer for 2 minutes. Speed of the screw was 65 rpm. Temperatures for P(CL30/LLA70), PEG-P(CL30-LLA70), PEG-P(CL30-DLLA70) and PEG-P(CL15-DLLA85) during extrusion were 145 °C, 135-140 °C, 125°C and 95-100°C respectively.

After extrusion, materials were compression molded (Fortune TB 400, Holland). Maximum weight of 5 grams of sample material were weighted for the mold. Preheating was 5 minutes long and so was the actual compression. 150 kN pressure were used. Parameters during extrusion and compression molding are found in *Table 2*.

***Table 2.*** *Processing and compression molding temperatures of polymers.*

| Material | Temperature during extrusion (°C) | Compression molding temperature (°C) | Cooling time (s) |
|---|---|---|---|
| **P(CL30/LLA70)** | 135-140 | 145 | 30 |
| **PEG-P(CL30-LLA70)** | 145 | 155 | 45 |
| **PEG-P(CL30-DLLA70)** | 125 | 125 | 10 |
| **PEG-P(CL15-DLLA85)** | 95-100 | 100 | 10 |

Maximum number of samples that were possible to make at once were 100. Shape of the samples were cylinders with 5 mm diameter and height approximately 2 mm. Compression molding was done in Aalto university (Espoo, Finland) by Sanja Asikainen.

For porous samples, supercritical carbondioxide ($sCO_2$) was used to achieve porous structure. Processing was done using high pressure and temperature in presence of $CO_2$. Processing method does not contain any toxic solvents which makes it tissue friendly (Davies et al. 2008). $sCO_2$ processing was done in Tampere University of technology (Tampere, Finland) by Kaarlo Paakinaho.

### 4.2.2 Inherent viscosity

Capillary viscosimetry was used for analyzing inherent viscosities. Measurements were done using Lauda capillary viscometer (Lauda-Königshofen, Germany) with Ubbelohde capillars (Schott-Instrument, Mainz, Germany) with chloroform as solvent at 25 °C. Results were used to predict processing parameters when samples were $CO_2$-processed. Additionally, results were used to compare drug release and how viscosity affects to that, even though viscosity is not proper parameter because polymer matrix does not flow like liquid (Siegel & Rathbone 2012). Two parallel samples were used for the samples without any drug. Sample sizes were around 20mg.

### 4.2.3 Size-exclusion cromatography

Size-exclusion chromatography (Water Associates system equipped with a Waters 717plus autosampler with waters 510 HPLS solvent pump and four linear gel columns ($10^4$, $10^5$, $10^3$ and 100 Å) connected to series and Waters 2412 differential refractometer) were used to measure molecular weights.

Number of parallel of samples was 2, except for samples containing drug it was 1. Polystyrene standards were used for calibration. SEC was used to measure Number average ($M_n$) and weight average ($M_w$) molecular weights and polydispersities (PD) of the samples after compression molding. Also samples processed with supercritical $CO_2$ without drugs were analyzed. Chloroform was used as solvent and eluent. Measurements were done in Aalto university (Espoo, Finland) by Sanja Asikainen.

### 4.2.4 Ultraviolet/visible-spectrophotometer

In this work Unicam UV 500 was used (Thermo Spectronic, Cambridge, England) with 1cm cuvette and Sörensen phosphate buffer solution or chloroform as solvent. Analysis was done to measure released drug from in vitro samples. Also initial drug contents were measured by dissolving samples in chloroform. For each different drug and solvent, calibration curves were ran. Released drug were calculated using following equation:

$$Released\ drug\ [{}^{\mu g}/_{mg}] = \frac{cv}{m} \tag{2}$$

where, c is measured concentration, v is volume of buffer solution and m is mass of measured sample.

For measuring the initial content of drug, approximately 20 mg dexamethasone samples were dissolved in 50 ml of chloroform. Parallel of samples were 5. Standard lines were determined for both drugs in solvent. For ascorbic acid, standard line in water was y=0.0334x ($R^2$=0.999, λ=260nm, n=11) and for dexamethasone in chloroform y=0.0347x ($R^2$=0.9979, λ=246nm, n=9).

Standard lines were done for both drugs in Sörensen phosphate buffer solution. For both drugs, plot remained linear up to 40 µg/mg concentration. In higher concentrations, the curve did not remain linear. Measured standard lines are found in *Figure 19* for dexamethasone and *Figure 20* for ascorbic acid salt.

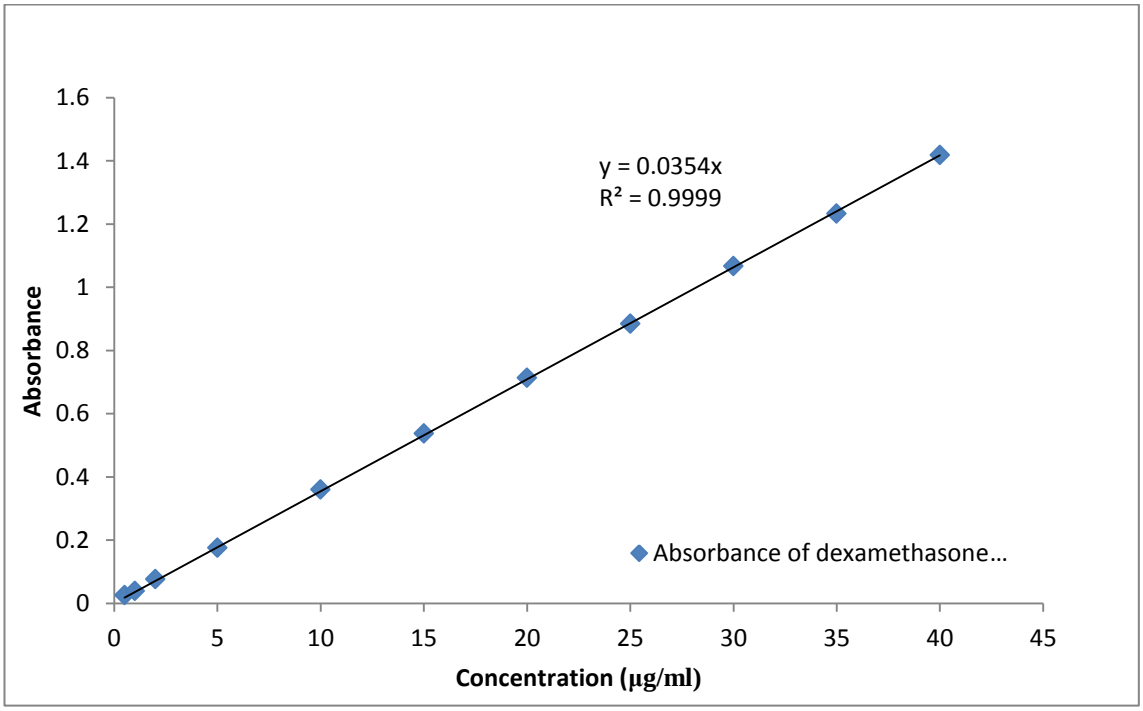



***Figure 19.*** *Standard line of dexamethasone in buffer solution (n=11).*

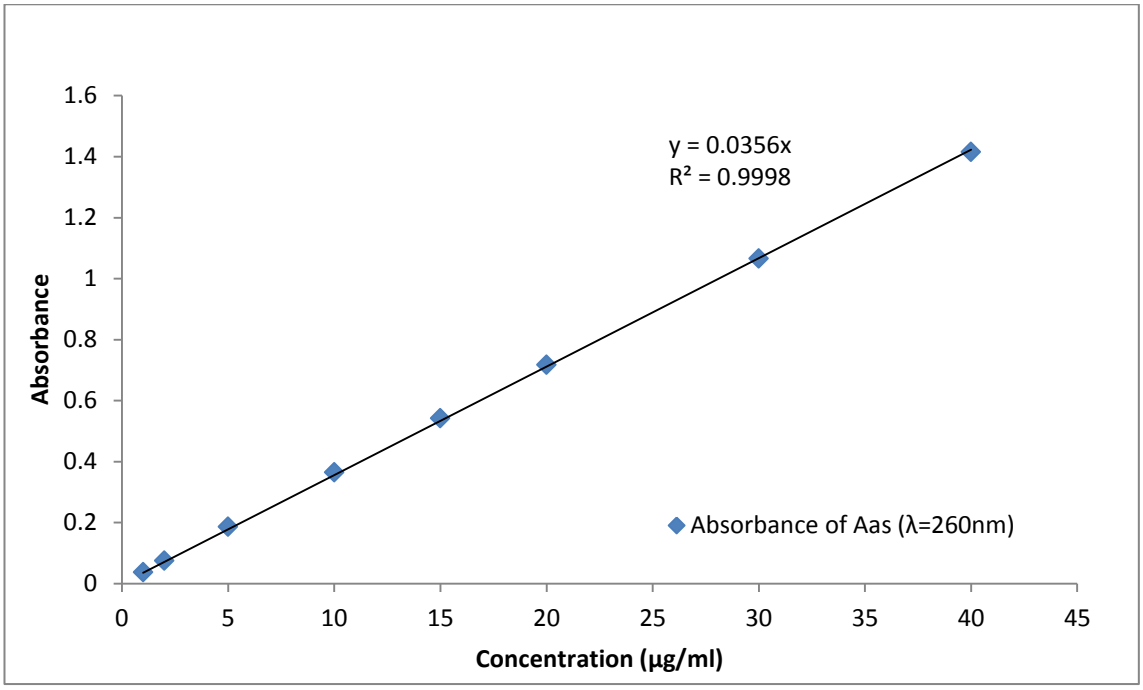



***Figure 20**. Standard line for AAs in buffer solution (n=9).*

Measurements of different scans were done in range of 190-600 nm and values in the peaks of curves were used for standard lines. For dexamethasone measurements got highest values at 241nm and for AAs at 260nm.

### 4.2.5 In vitro drug release test series

Six parallel test series were started by weighting samples using analytical scale (Mettler Toledo, AG 245) after storing them in vacuum at least 3 days. 10 ml of Sörensen buffer (pH 7.4, prepared using ISO-15814 standard) was measured to each brown glass bottle with samples. These were kept in shaking incubator at 37 °C and 100 rpm. Measurements of released drug was done using UV/VIS-spectrophotometry scanning samples in range of 190-450 nm, to make sure that there was no air bubbles to interfere measurements.

### 4.2.6 Microcomputed tomography

Microcomputed tomography (µCT) was used for visual examination of porous samples. Also porosity, mean pore size and standard deviation and area of sample were analyzed. µCT imaging (Carl Zeiss X-ray Microscopy Inc., Pleasanton, CA, USA) was done using 80 kV source voltage, 125 µA source current and 6.2µm voxel size. Reconstruction was done using Xradia's XMReconstructor software. For manual image segmentation was used a Fiji, an opensource software. Analysis was done using same software with BoneJ

plugin. All imaging and image analysis was done in Tampere university of technology (Tampere, Finland) by Markus Hannula.

### 4.2.7 Thermal analysis

Thermal analysis was done using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). DSC was used to measure glass transition temperatures ($T_g$) and melting temperatures ($T_m$). Five parallel samples were used due to relatively high standard deviation of samples. DSC run was done using DSC Q1000 (TA Instruments, Delaware, USA) under nitrogen. Two heating scans were made (20°C/min) from -20°C to 200°C with 1 minute stand at 200°C and cooling at rate of -50°C/min. $T_g$s were analyzed from second heating and $T_m$s from the first. For the analysis of the results, TA Universal analysis software was used.

Due to high melting point of both drugs and lower decomposion of polymers used, heat could not rise high enough to see if there is going to be a melting peak or not. It would tell whether the drug is dissolved or dispersed in polymer matrix. It was decided to run thermogravinometric analysis to get possibly some information of thermal behavior near drug melting point. Analysis was done by heating at rate of 20°C/min up to 600 °C under air atmosphere. TGA 500 (TA Instruments, Delaware, USA) were used for measuring and analysis were done using same software as were used in analyzing DSC samples. Only one sample was ran for each polymer-drug combination.

# 5 RESULTS AND DISCUSSION

## 5.1 Molecular weight

Weight average ($M_w$) and number average ($M_n$) molecular weights of porous and non-porous samples without drug are listed with the measured polydispersities (PD) in *Table 3*. It seems that processing samples with supercritical $CO_2$ affects by reducing molecular weights slightly. However, GPC as an analysis method is not very accurate and differences of few thousand g/mol may not be significant. Samples were tested after processing, before starting drug release test series.

***Table 3**. Measured $M_w$ and $M_n$ for porous and nonporous polymer samples (n=2).*

| | Solid samples | | | Porous samples | | |
|---|---|---|---|---|---|---|
| **Polymers** | $M_w$ | $M_n$ | PD | $M_w$ | $M_n$ | PD |
| **P(CL30-LLA70)** | 235000 | 142000 | 1.65 | 232000 | 140000 | 1.66 |
| **PEG-P(CL30-LLA70)** | 78000 | 50000 | 1.56 | 65000 | 45000 | 1.44 |
| **PEG-P(CL30-DLLA70)** | 152000 | 88000 | 1.73 | 138000 | 92000 | 1.50 |
| **PEG-P(CL15-DLLA85)** | 220000 | 147000 | 1.50 | 157000 | 97000 | 1.62 |

Molecular weights of solid samples with drugs are listed in *Table 4*.

***Table 4.*** *Measured $M_w$, $M_n$ and PD values of used polymer-drug combinations (n=1) (A=ascorbic acid salt, D=dexamethasone and 4,8=weight contents of drugs).*

| Material | $M_w$ | $M_n$ | PD |
|---|---|---|---|
| **P(CL30-LLA70)** | 235000 | 142000 | 1.65 |
| **A4** | 225000 | 142000 | 1.58 |
| **A8** | 201000 | 128000 | 1.57 |
| **D4** | 233000 | 139000 | 1.68 |
| **D8** | 230000 | 136000 | 1.69 |
| **PEG-P(CL30-LLA70)** | 78000 | 50000 | 1.56 |
| **A4** | 67000 | 40000 | 1.68 |
| **A8** | 53000 | 31000 | 1.71 |
| **D4** | 84000 | 57000 | 1.47 |
| **D8** | 82000 | 54000 | 1.52 |
| **PEG-P(CL30-DLLA70)** | 152000 | 88000 | 1.73 |
| **A4** | 130000 | 83000 | 1.57 |
| **A8** | 122000 | 80000 | 1.53 |
| **D4** | 145000 | 92000 | 1.58 |
| **D8** | 149000 | 95000 | 1.57 |
| **PEG-P(CL15-DLLA85)** | 220000 | 147000 | 1.50 |
| **A4** | 206000 | 141000 | 1.46 |
| **A8** | 177000 | 103000 | 1.72 |
| **D4** | 210000 | 139000 | 1.51 |
| **D8** | 227000 | 153000 | 1.48 |

With dexamethasone samples, molecular weights remains almost at same level except there is small increase with PEG-P(CL30-LLA70). Decrease in molecular weights was observed for samples containing ascorbic acid salt derivative. PD values are in between 1.46 and 1.73. Molecular weights were expected to have effect to degradation of materials and release of drugs.

## 5.2 Inherent viscosity

Measured inherent viscosities are in *Table 5*. Measured molecular weighs are well in consistent with measured i.v results.

***Table 5**. i.v results of used polymers. (n=2)*

| Material | Viscosity (dl/g) |
|---|---|
| **P(CL30-LLA70)** | 0.99 |
| **PEG-P(CL30-LLA70)** | 0.76 |
| **PEG-P(CL30-DLLA70)** | 1.29 |
| **PEG-P(CL15-DLLA85)** | 1.56 |

PEG-P(CL30-LLA70) has the smallest measured i.v. value (0.76 dl/g) and molecular mass ($M_w$ of 78 000g/mol) compared to other results. Also release rates were fastest for this material compared to corresponding combinations of other materials. Drug molecules were released easily from this material. However, low viscosity and molecular mass was not probably only reason why drug release was fast and did not dominate release. Ethylene glycol incorporation into polymer backbone has made it more hydrophilic than commercial P(CL30-LLA70). Especially AAs has easy escape from material. General porosity were smallest however.

PEG-(CL15-DLLA85) has $M_w$ of 220 000 g/mol and i.v. 1.56 g/mol but dexamethasone release was faster than from PEG-(CL30-DLLA70) which did not have that high values ($M_w$ of 152 000 g/mol and i.v. 1.29 g/mol). Inherent viscosity does not explain difference here. Reason could be better permeability caused by higher caprolactone-content of copolymer PEG-(CL30-DLLA70). Release rate of AAs from PEG-(CL15-DLLA85) was slightly higher than from PEG-(CL30-DLLA70).

## 5.3 Stability of drugs

Stability test was done to both of the drugs. Solution of drug and buffer solution (40µg/ml) was prepared and stored in a refrigerator (2 ˚C) and shaking incubator (37 ˚C, 100 rpm). Dexamethasone containing samples were placed in test tubes and sealed with rubber corks. It was noticed that used corks did not manage to keep evaporated solution inside container. This was seen as increase in measured absorbance. Test tubes were decided change to brown 25ml bottles with screw caps which were used already in measuring AAs samples. For AAs, some changes in concentration were seen during one week test period. For cold stored samples, concentration decreased 0.8 % and for incubator stored 4,8% from initial drug content of prepared solution.

## 5.4 Initial drug content

The solutions were measured using UV/VIS-spectrometer. Pipetting of chloroform was difficult due to low viscosity that makes it leaking out of pipette. Additionally, when initial drug contents were measured, lamps of UV/VIS-spectrometer started to lose their power. This could possibly have an effect to measurements.

There was not found any significant difference in dexamethasone contents between solid and porous samples. However all samples included less drug that theoretically there should have been. It is possible that some of the drug was left to feeder during blending but likely it is not the only reason why some are missing. Drug may have destroyed in processing because of heat for example. With PEG-P(CL15-DLLA85) samples that should have contained 8-wt% dexamethasone, the standard deviations were relatively high. It was 3.93 for solid samples and 3.20 for porous samples. It is interesting since dexamethasone blended well with used polymers. Possibly polymer may have become saturated from drug and the rest of the drug is in dispersed form. Measured initial contents of dexamethasone containing samples are found from *Table 6*.

***Table 6.*** *Measured drug content of dexamethasone (n=5).*

| Material | Theoretical content (%) | Average drug content (%) | Standard deviation | Average drug content in porous samples(%) | Standard deviation of porous samples |
|---|---|---|---|---|---|
| **P(CL30-LLA70)** | 4 | 3.48 | 0.12 | 3.50 | 0.11 |
| | 8 | 6.30 | 0.22 | 6.48 | 0.10 |
| **PEG-P(CL30-LLA70)** | 4 | 3.66 | 0.26 | 3.45 | 0.25 |
| | 8 | 6.53 | 0.10 | 6.61 | 0.11 |
| **PEG-P(CL30-DLLA70)** | 4 | 3.30 | 0.49 | 3.38 | 0.66 |
| | 8 | 7.07 | 0.04 | 7.48 | 0.18 |
| **PEG-P(CL15-DLLA85)** | 4 | 3.71 | 0.09 | 4.06 | 0.13 |
| | 8 | 7.25 | 3.93 | 9.57 | 3.20 |

Because AAs is soluble only into water and some organic solvents, drug content was chosen to be measured by using extraction. Used solvents were chloroform and distilled water. Solubility of AAs into chloroform should be negligible. there is not much data available about solubility's of ascorbic acid deviations, but ascorbic acid is insoluble in many organic solvents including chloroform (Anon 2012). Extraction was done by first dissolving samples (n=5) into 20 ml of chloroform. Then the solution was poured into a separating funnel. Measuring flask was rinsed with distilled water to get all drug to funnel. Extraction was done tree times. Results of initial drug contents of ascorbic acid salt containing samples are found from *Table 7*.

***Table 7**. Measured drug content of AAs (n=5).*

| Material | Theoretical content (%) | Average drug content (%) | Standard deviation | Average drug content in porous samples(%) | Standard deviation of porous samples |
|---|---|---|---|---|---|
| **P(CL30-LLA70)** | 4.00 | 3.11 | 0.41 | 2.80 | 0.39 |
| | 8.00 | 6.95 | 0.75 | 6.62 | 0.31 |
| **PEG-P(CL30-LLA70)** | 4.00 | 1.44 | 0.25 | 3.62 | 1.69 |
| | 8.00 | 4.26 | 1.14 | 3.82 | 0.49 |
| **PEG-P(CL30-DLLA70)** | 4.00 | 2.11 | 0.41 | 2.31 | 0.32 |
| | 8.00 | 5.46 | 0.56 | 5.52 | 1.27 |
| **PEG-P(CL15-DLLA85)** | 4.00 | 3.44 | 0.49 | 3.02 | 0.58 |
| | 8.00 | 7.20 | 0.44 | 6.44 | 1.10 |

Again with every polymer-drug combination, measured contents were less than theoretical ones. Also drug contents did not change much during $CO_2$-processing. There were some combinations where standard deviations were relatively high. Results of PEG-P(CL30-LLA70) 4-wt% including ascorbic acid salt were interesting. For solid samples, measured drug content was 1.44-wt% and for porous it was 3.62. This was the same material which processing temperature was highest causing samples turning into yellowish. It was thought that some kind of reaction may have happened. However, with these measured drug contents, the actual drug release profiles seems more reasonable than using theoretical contents.

Measuring AAs content was a bit problematic since there was some white thick precipitation present at measurements. It appeared either on water or chloroform phase and no logic was found in the behavior. There were differences between parallel samples too. Use of higher solvent volume could have helped on this issue. However, the results seemed reasonable.

## 5.5 Drug release of dexamethasone

Drug concentration of the buffer solution was followed periodically, at least once a week. After measurement, the whole buffer solution was changed to fresh. From meas-

ured absorbance and known mass of sample and volume of buffer, the released amount of drug was calculated by using Matlab software. Calculations were done using equation (2). Cumulative releases were calculated from sums of measured released drugs and percentage release by dividing former result by initial drug content. Cumulative drug release from materials of P(CL30-LLA70), PEG-P(CL30-LLA70), PEG-P(CL30-DLLA70) and PEG-P(CL15-DLLA85) are presented in *Figure 21*, *Figure 22*, *Figure 23* and *Figure 24* respectively. Release curves are presented with error bars that are equal to standard deviations. p before abbreviations means that samples were porous and numbers 4 and 8 denote weight per cents of drug in the samples.

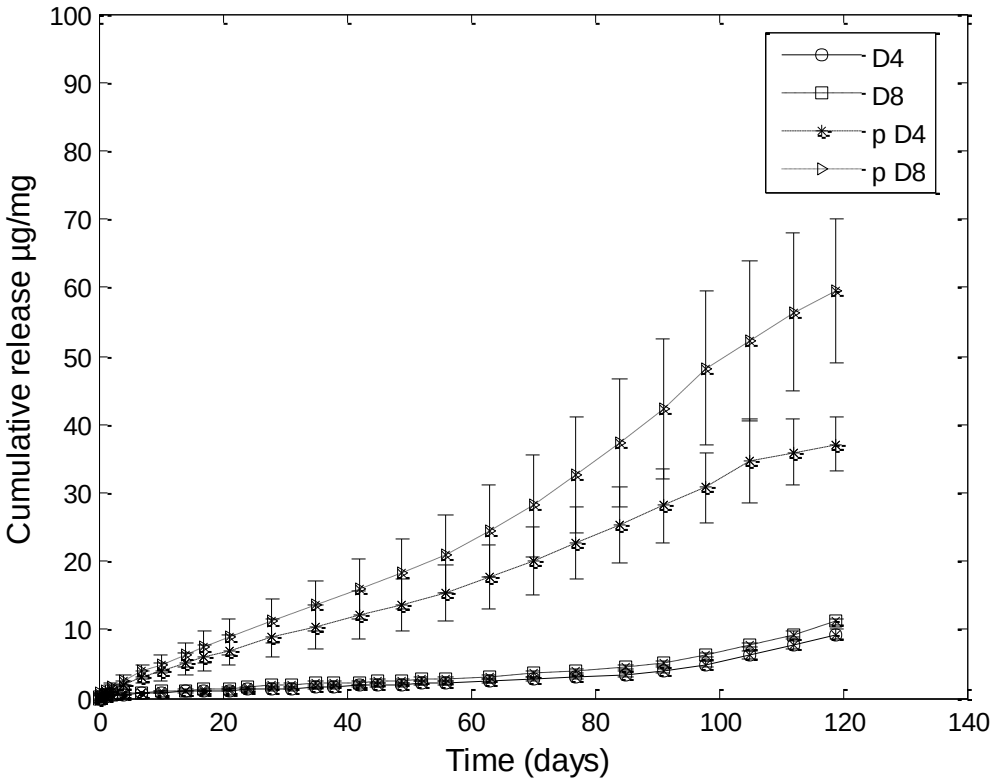



***Figure 21.*** *Cumulative dexamethasone release from P(CL30-LLA70) where p is used to mark porous samples, D means that sample includes dexamethasone, and number after D is theoretical drug content.*

Release of dexamethasone from solid P(CL30-LLA70) was very slow during 120 days in vitro test series (*Figure 21*). It looks like the degradation and erosion of matrix was controlling the release of dexamethasone. From porous samples the release kinetics was quite close to zero-order kinetics.

*Figure 22* presents release of dexamethasone from PEG-P(CL30-LLA70). Lot of similarities can be found compared to the commercial material. Here also, the drug is released quite steadily from solid samples. Slight difference in release profile is possible to see and rates are slightly higher.

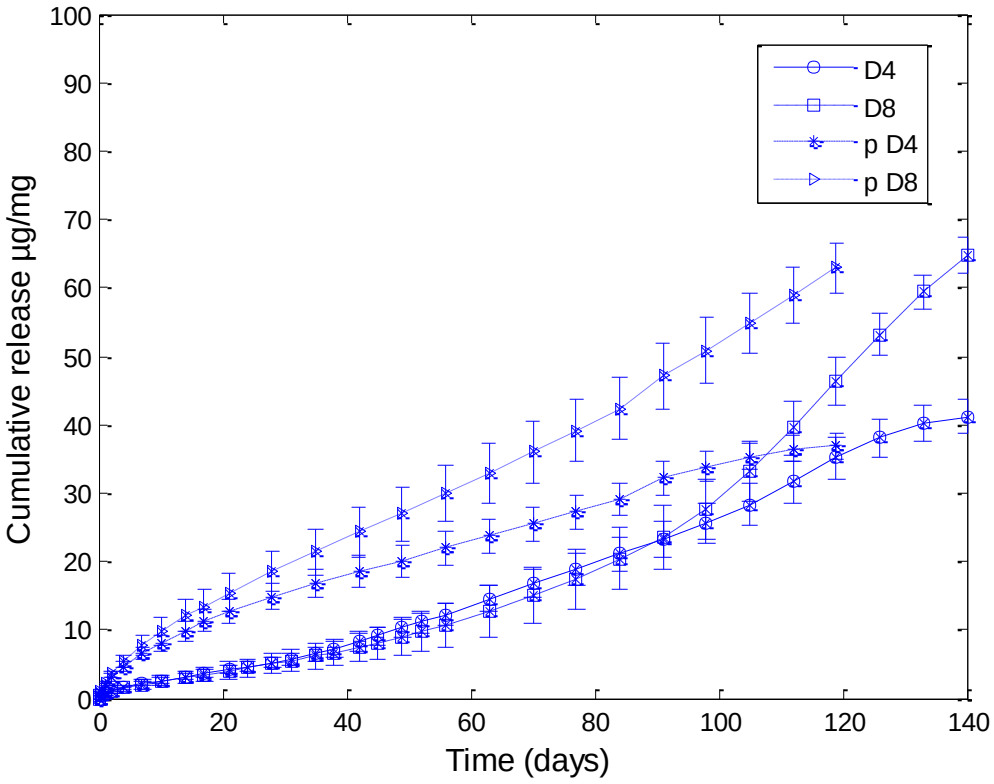



***Figure 22.*** *Cumulative dexamethasone release from PEG-P(CL30-LLA70) where p is used to mark porous samples, D means that sample includes dexamethasone, and number after D is theoretical drug content.*

When type of lactide monomer was changed from D-lactide to DL-lactide, the release pattern became very different than it was with PEG-P(CL30-LLA70) compared to PEG-P(CL30-DLLA70). Release profiles are presented in *Figure 22* and *Figure 23* respectively. There is a relatively long lag period before release starts. It looks like the release occurs by erosion of the material. Solid samples degraded relatively fast because of autocatalysis. Measurements were finished when samples were so degraded that measurements would not be reliable anymore. Solution did not look homogenous anymore. It became blurry after breakdown of samples. Basically shells of polymers were left when measurements were finished, which supports the suggestion of autocatalysis.

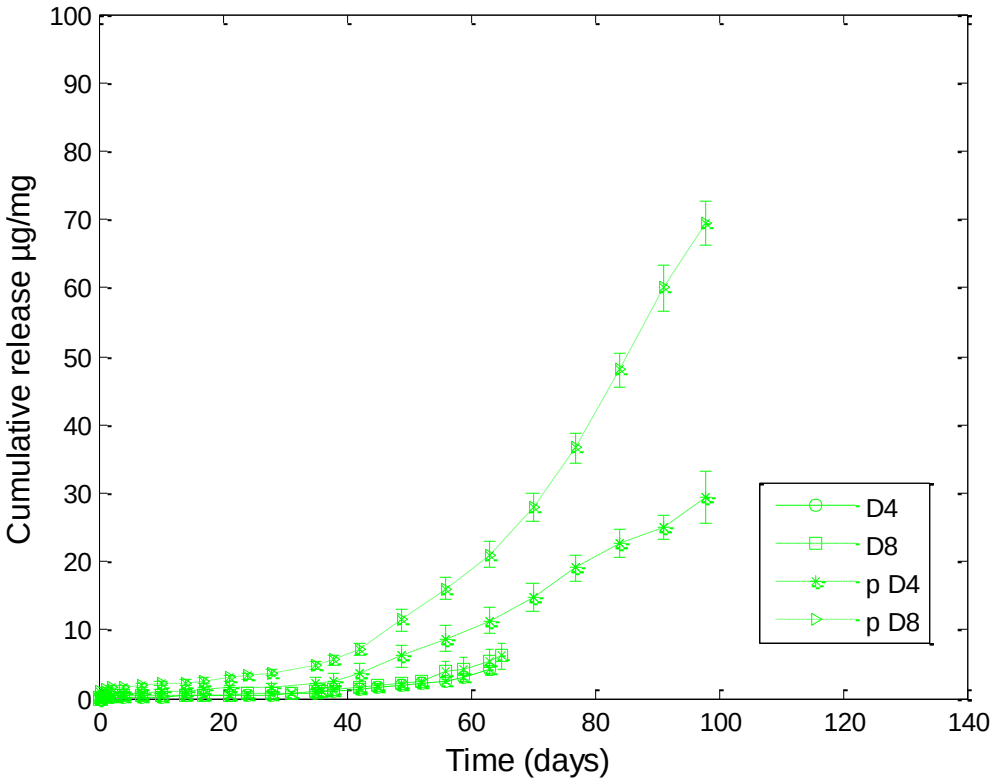



***Figure 23.*** *Cumulative dexamethasone release from PEG-P(CL30-DLLA70) where p is used to mark porous samples, D means that sample includes dexamethasone, and number after D is theoretical drug content.*

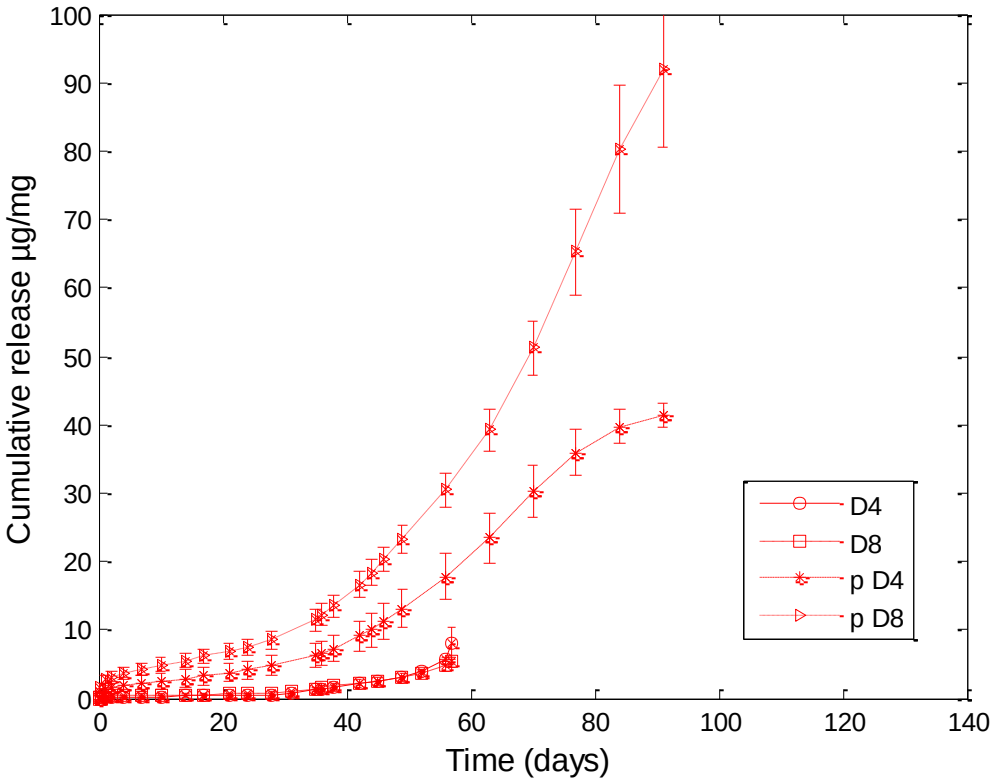



***Figure 24.*** *Cumulative dexamethasone release from PEG-P(CL15-DLLA85) where p is used to mark porous samples, D means that sample includes dexamethasone, and number after D is theoretical drug content.*

Last polymeric material was PEG-P(CL15-DLLA85). LA-content was raised from 70% to 85%. Release profile (*Figure 24*) looks very similar, but the release rate is slightly higher than in PEG-P(CL30-DLLA70). With solid samples, measurements had to end before 2 months were past because fast degradation. Samples degraded in similar way than PEG-P(CL30-DLLA70) containing dexamethasone samples.

From *Figure 25* and *Figure 26* is possible to compare measured results of dexamethasone samples while cumulative release (%) is shown as function of time. Fitting is done using measured initial values of dexamethasone.

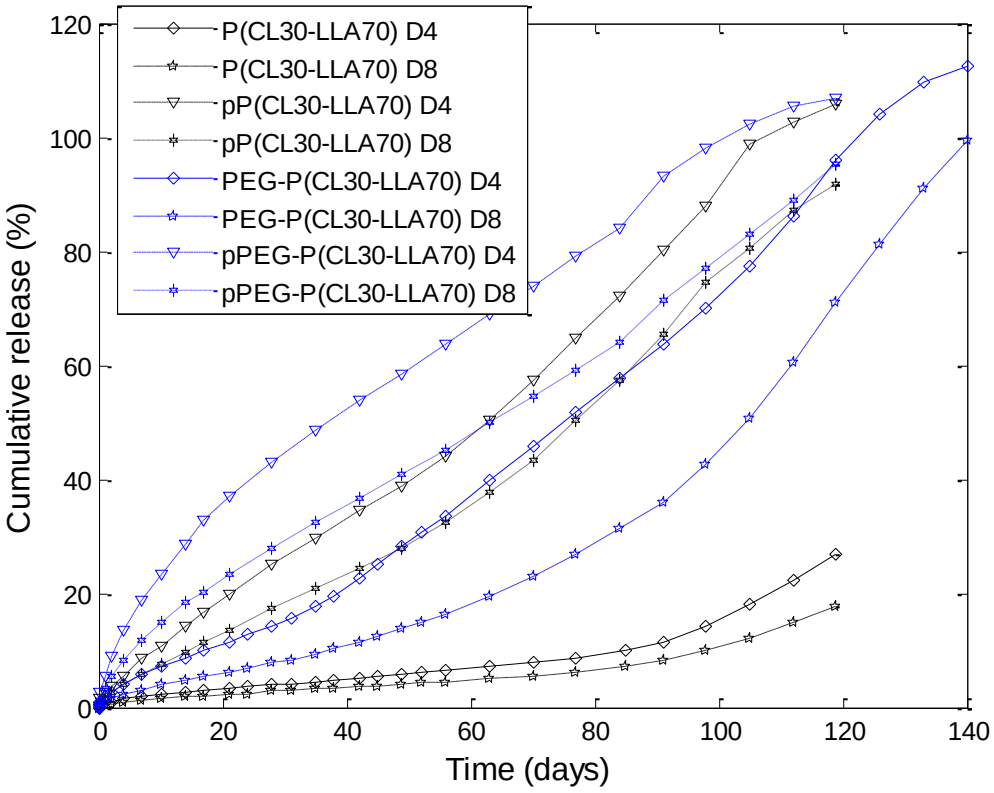



***Figure 25.*** *Cumulative release of DEX from P(CL30-LLA70 and PEG-(CL30-LLA70) where p is used to mark porous samples, D means that sample includes dexamethasone, and number after D is theoretical drug content.*

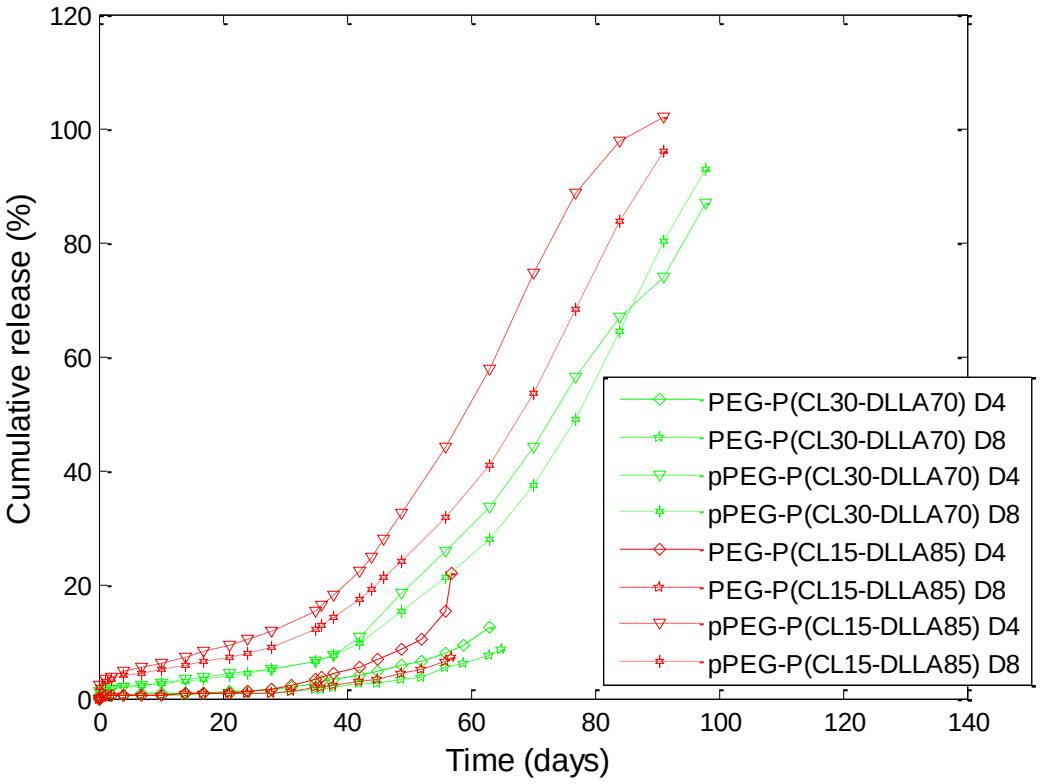



***Figure 26.*** *Cumulative release of DEX from PEG- P(CL30-DLLA70 and PEG-(CL15-DLLA85) where p is used to mark porous samples, D means that sample includes dexamethasone, and number after D is theoretical drug content.*

## 5.6 Drug release of ascorbic acid salt

Release of AAs from polymers was very different than dexamethasone release from the same polymers. This was probably due to different properties like solubility of drugs. All measuring and calculating was done in similar way than for dexamethasone containing samples. Cumulative ascorbic acid salt release from materials of P(CL30-LLA70), PEG-P(CL30-LLA70), PEG-P(CL30-DLLA70) and PEG-P(CL15-DLLA85) are presented in *Figure 27*, *Figure 28*, *Figure 29* and *Figure 30* respectively. Release curves are presented with error bars that are equal to calculated standard deviations.

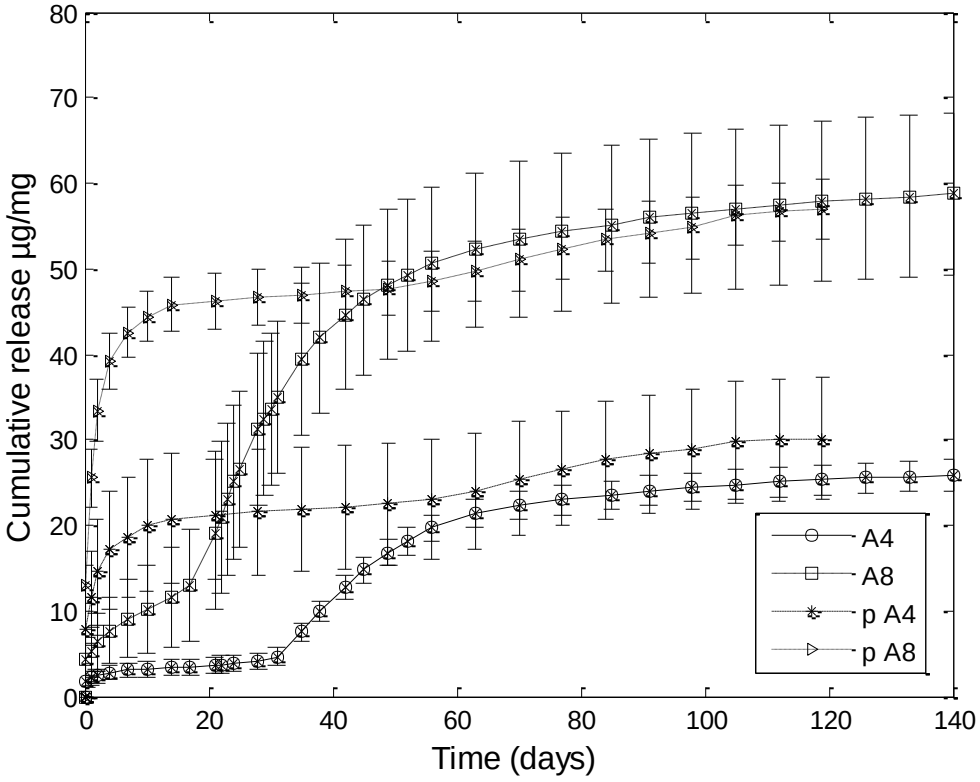



***Figure 27.*** *Cumulative AAs release from P(CL30-LLA70) where p is used to mark porous samples, A means that sample includes ascorbic acid salt, and number after A is theoretical drug content.*

In *Figure 27* drug release from commercially available material is presented. Release profiles differ from others seen in this work. There are different phases in the release. At this point it would be useful to know better degradation behavior of P(CL30-LLA70). For solid samples, the release started from burst following a lag period. For higher AAs content drug, there was some release during the "lag period". Faster release starts approximately at 20 day timepoint and for 4-wt% samples fast release starts approximately 30 day timepoint. Burst effect is seen with all combinations. It looks that it is proportional to the drug content. Burst is higher with porous samples compared to solid ones. This is probably due to the fact that the drug has easier path to solution due to higher surface area.

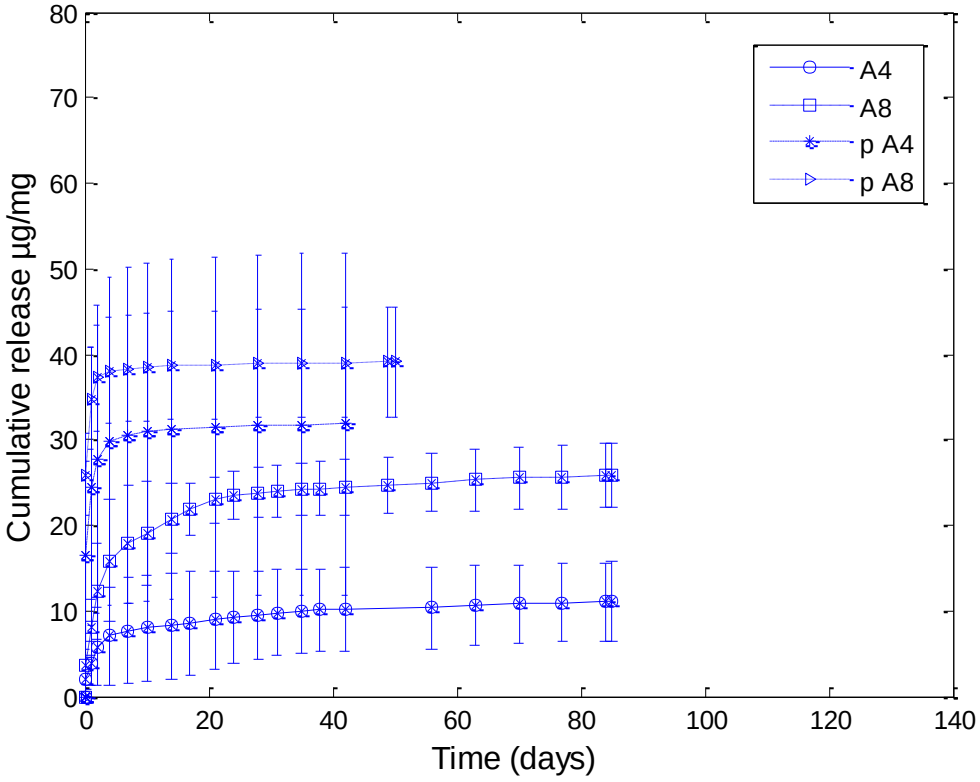



***Figure 28.*** *Cumulative AAs release from PEG-P(CL30-LLA70) where p is used to mark porous samples, A means that sample includes ascorbic acid salt, and number after A is theoretical drug content.*

It seems that adding PEG into the polymer backbone changed the release profile to obeying mostly first order kinetics. This was the material that was thought to react somehow with the drug during processing. Cumulative release curves are found from *Figure 28* for PEG-P(CL30-LLA70). Burst effect is relatively high also here especially with porous samples. Again, it looks like drug content is proportional to burst effect. Almost all drug is released form 8-wt% samples during first days.

When L-lactide was changed to DL-lactide (*Figure 29*), changes were seen in the release profile. With these amorphous materials, the release test had to be ended when samples were too degraded and measurements would not been reliable anymore. From solid samples, the release was almost negligible before material degraded. With porous samples, almost all drug was released rapidly.

When LA/CL ratio is increased from 70/30 to 85/15 (*Figure 30*), profiles are really similar. However it looks like increasing LA-content increases release rates slightly. Burst release however looks slightly smaller.

Both materials, PEG-P(CL30-DLLA70) and PEG-P(CL15-DLLA85) was kept in hydrolysis one week longer than same ones including dexamethasone. The different nature of drug was probably reason why DEX-samples degraded faster.

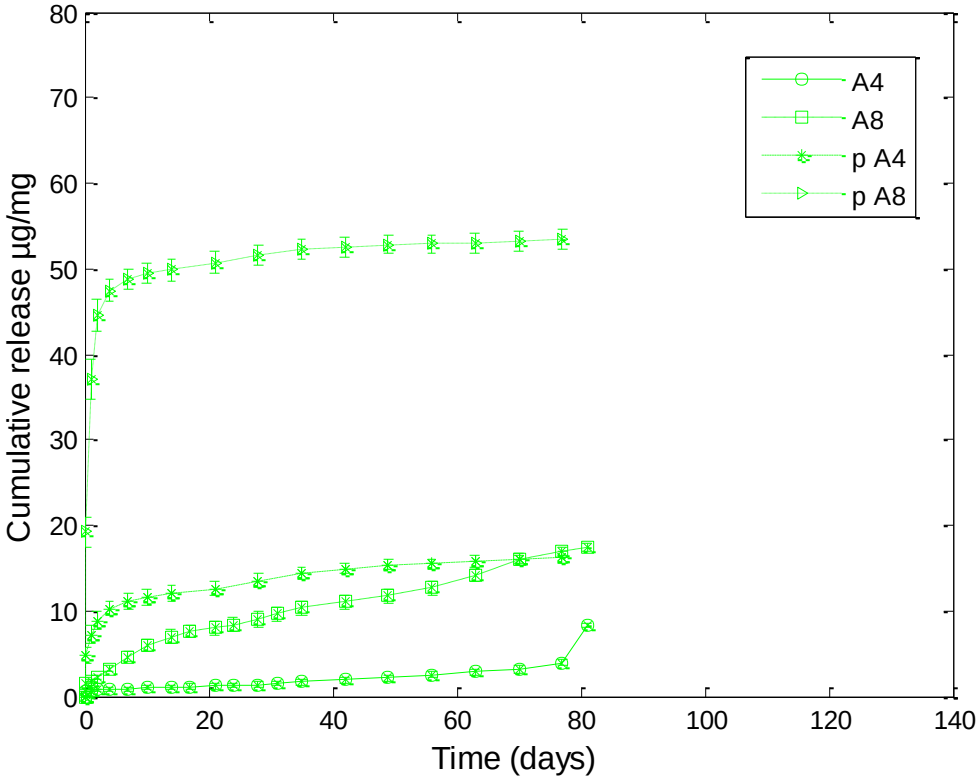



***Figure 29.*** *Cumulative AAs release from PEG-P(CL30-DLLA70) where p is used to mark porous samples, A means that sample includes ascorbic acid salt, and number after A is theoretical drug content.*

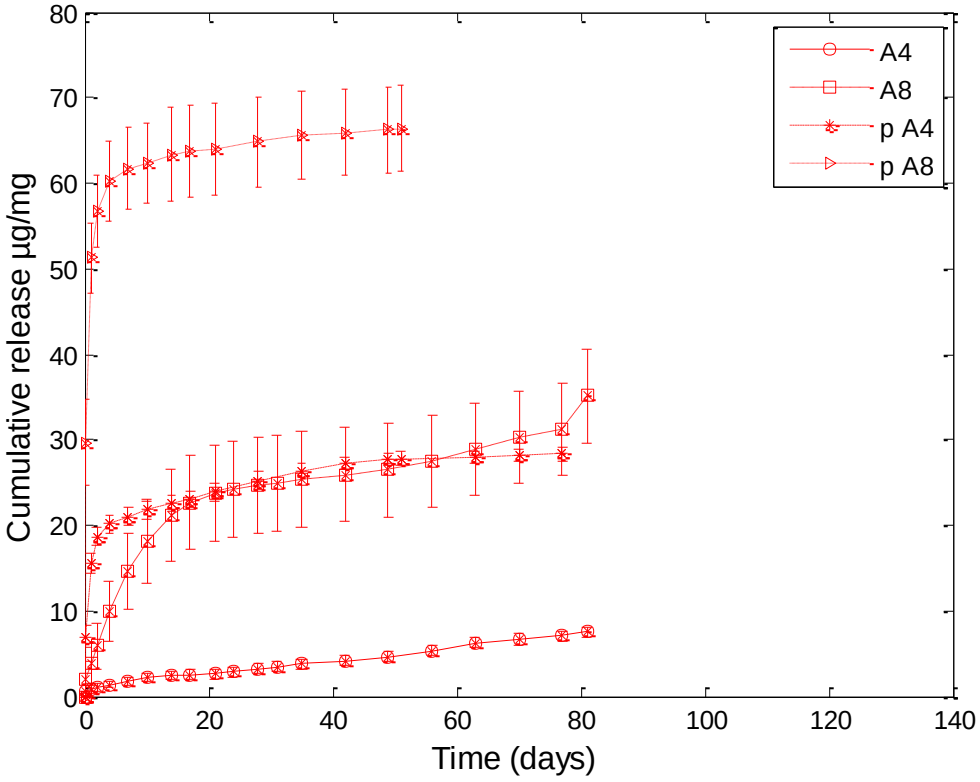



***Figure 30.*** *Cumulative AAs release from PEG-P(CL15-DLLA85) where p is used to mark porous samples, A means that sample includes ascorbic acid salt, and number after A is theoretical drug content.*

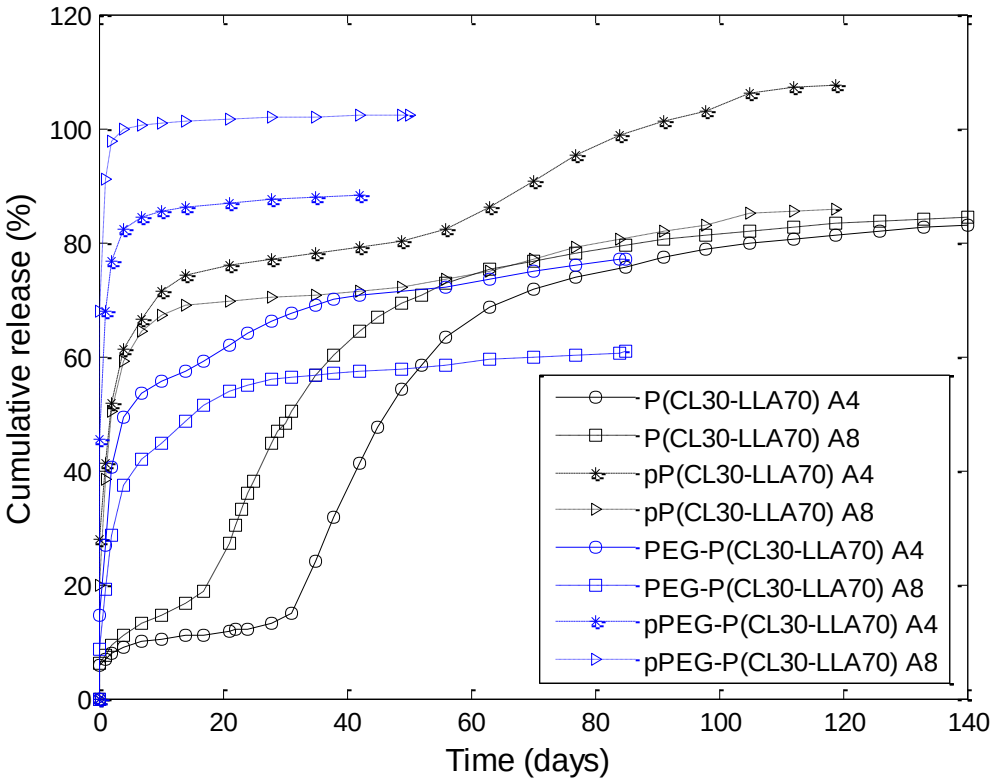



***Figure 31.*** *Cumulative release of AAS from P(CL30-LLA70) and PEG-P(CL30-LLA70) where p is used to mark porous samples, A means that sample includes ascorbic acid salt, and number after A is theoretical drug content.*

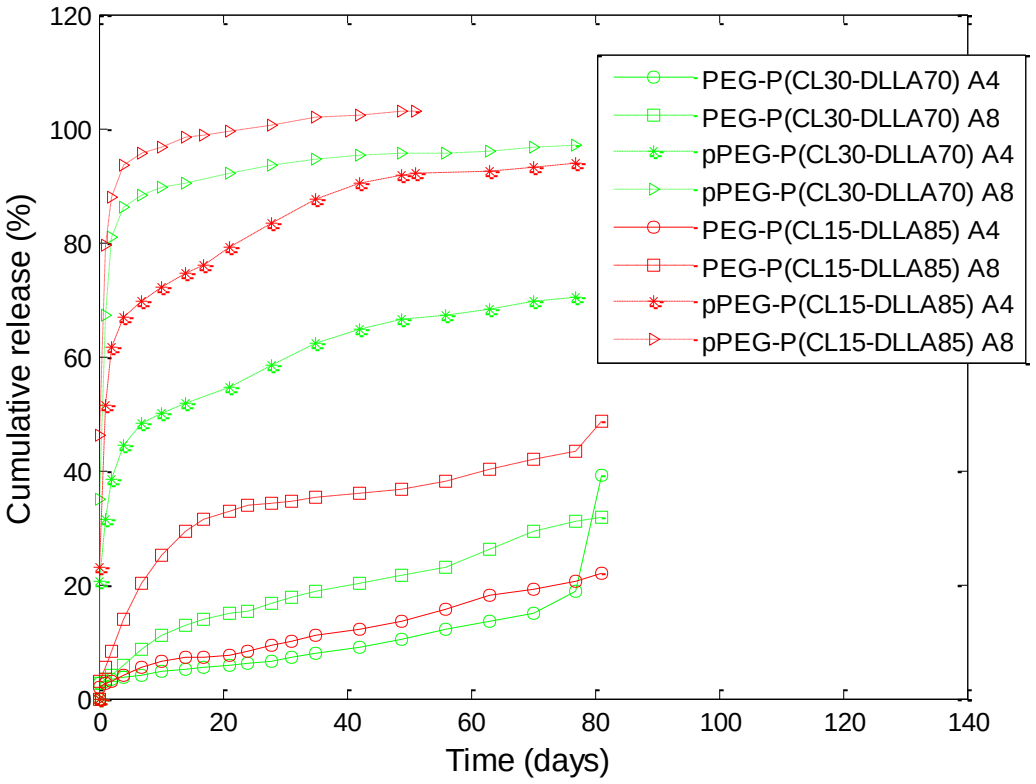



***Figure 32.*** *Cumulative release of AAS from PEG-P(CL30-DLLA70).and PEG-P(CL15-DLLA85) where p is used to mark porous samples, A means that sample includes ascorbic acid salt, and number after A is theoretical drug content.*

In *Figure 31* and *Figure 32* release profiles were scaled to maximum drug amount (100%). Maximum values are based on results from initial drug content measurements. Patterns looks more clear in these figures. With commercial samples it is interesting to notice that release after burst and lag phase starts more than a week later in 4-wt samples. Concentration gradient is bigger with higher drug content, but the drug may have effected to whole release system in a way, that was seen in earlier start of drug release. For example present drug may change the system to be more hydrophilic.

Burst release was present with all AAs samples and was relatively high. With commercial samples, a lag period was seen after burst. Yoon et al. (2003) suggested in their work that lag after burst release of drug was possibly caused by temporal shortage of drug for diffusion. Burst also increased while drug contents were increased. Similar was observed by Grinberg et al. (2010) in their study. It was suggested that higher force of diffusion caused higher burst at beginning.

Zhang et al. (2004) prepared PEG-b-P(CL-co-LLA) nanoparticles with Camptothecin derivative, a poorly water soluble cancer drug. It was suggested that fast release was due to molecularly dispersed drug and because of low $T_g$ of used polymers. Low $T_g$ was considered to make permeability of drug better than in glassy matrix.

In study of Hu et al. (2003) also polymerized nanosized particles of lactide and caprolactone in presence of ethylene glycol. Chemical composition of particles had key

role in controlling of drug Nimodipine release. Size of used PEG-block was varied from 1000 g/mol to 20000 g/mol. Higher PEG-content led to higher release rate.

## 5.7 Differential scanning calorimetry

Results of DSC scans are shown in *Table 8*. $T_g$s were analyzed from second heating to make sure that samples had same thermal histories. $T_m$s were analyzed from the first heating cycle. It is interesting notice that no melting peak can be found for P(CL30-LLA70) which was expected to have some crystallinity in structure. Absence of crystallinity is probably because of fast cooling during processing.

It seems that with all materials, blending dexamethasone causes and increase in glass transition temperatures. And it looks like increase of drug is consistent with blended drug content. Because drug changes the glass transition, it probably has good interaction with polymers. PEG-P(CL15-DLLA85) makes an exception. For pure material $T_g$ was 28.57 °C and for 4-wt% dexamethasone samples it decreased to 28.14 °C but with 8-wt% samples it increases to 30.92 °C.

For ascorbic acid, effect is not that clear. Glass transition temperatures increases with samples of P(CL30-LLA70) and PEG-P(CL15-DLLA85), but decreases for PEG-P(CL30-LLA70) and remains almost at same level for PEG-P(CL30-DLLA70). When drug does not change glass transition temperature, it probably does not have good interaction with polymer matrix (Karjalainen et al. 2000). However decrease of glass transition temperature is usually connected to plastizising effect of drug (Yoon et al. 2003; Velasco et al. 2010).

***Table 8**. Results of DSC. For polymers without drug n=3 and polymers with drug n=5. A means ascorbic acid salt, D means dexamethasone and numbers 4 and 8 theoretical drug contents.*

| Sample | Tg (°C) | sd | Tm (°C) | sd |
|---|---|---|---|---|
| **P(CL30-LLA70)** | 23.07 | | | |
| A4 | 23.20 | 0.43 | | |
| A8 | 23.26 | 0.72 | | |
| D4 | 24.76 | 0.31 | | |
| D8 | 24.27 | 0.42 | | |
| **PEG-P(CL30-LLA70)** | 19.96 | | 144.91 | |
| A4 | 19.39 | 0.28 | 145.49 | 0.26 |
| A8 | 17.98 | 0.78 | 145.27 | 0.22 |
| D4 | 20.91 | 0.89 | 144.41 | 0.18 |
| D8 | 21.27 | 0.78 | 144.18 | 0.57 |
| **PEG-P(CL30-DLLA70)** | 35.24 | | | |
| A4 | 35.26 | 0.68 | | |
| A8 | 35.15 | 0.35 | | |
| D4 | 36.14 | 0.57 | | |
| D8 | 36.50 | 0.34 | | |
| **PEG-P(CL15-DLLA85)** | 28.57 | | | |
| A4 | 30.60 | 0.52 | | |
| A8 | 30.53 | 1.72 | | |
| D4 | 28.14 | 0.51 | | |
| D8 | 30.92 | 2.94 | | |

Melting peaks were possible to find only for PEG-P(CL30-LLA70) samples. For AAs containing samples, melting temperatures increased while dexamethasone containing samples had opposite effect.

For all analyzed results, the standard deviations were somewhat high even five parallel samples were used. Although, polymers were purified only once and it was noticed that AAs did not blend very well with used polymers.

As it was mentioned before, caprolactone has been favored in drug release applications due to its good permeability to drugs which is caused by low glass transition temperature. Measured results showed that PEG-P(CL30-LLA70) had lowest $T_g$. Glass transition of pure polymer without any drug was 19.96 °C. Tg of P(CL30-LLA70) was 23.07 °C, PEG-P(CL30-DLLA70) had 35.24 and PEG-P(CL15-DLLA85) had 28.57 °C. Last two polymers have relatively high glass transitions, especially PEG-P(CL30-DLLA70) which is relatively close to physiological temperature. These two polymers

had the lowest release rates with solid samples. Permeation of drugs was probably somewhat poor.

## 5.8 Thermogravimetric analysis

Thermogravimetric analysis was done once for each drug-polymer combinations (n=1). In *Figure 33* TGA curves for 8-wt% dexamethasone containing samples and in figure *Figure 34* same except for ascorbic acid salt are presented. In both cases, black line is for P(CL30-LLA70), blue for PEG-P(CL30-LLA70), green for PEG-P(CL30-DLLA70) and red for PEG-P(CL15-DLLA85) samples.

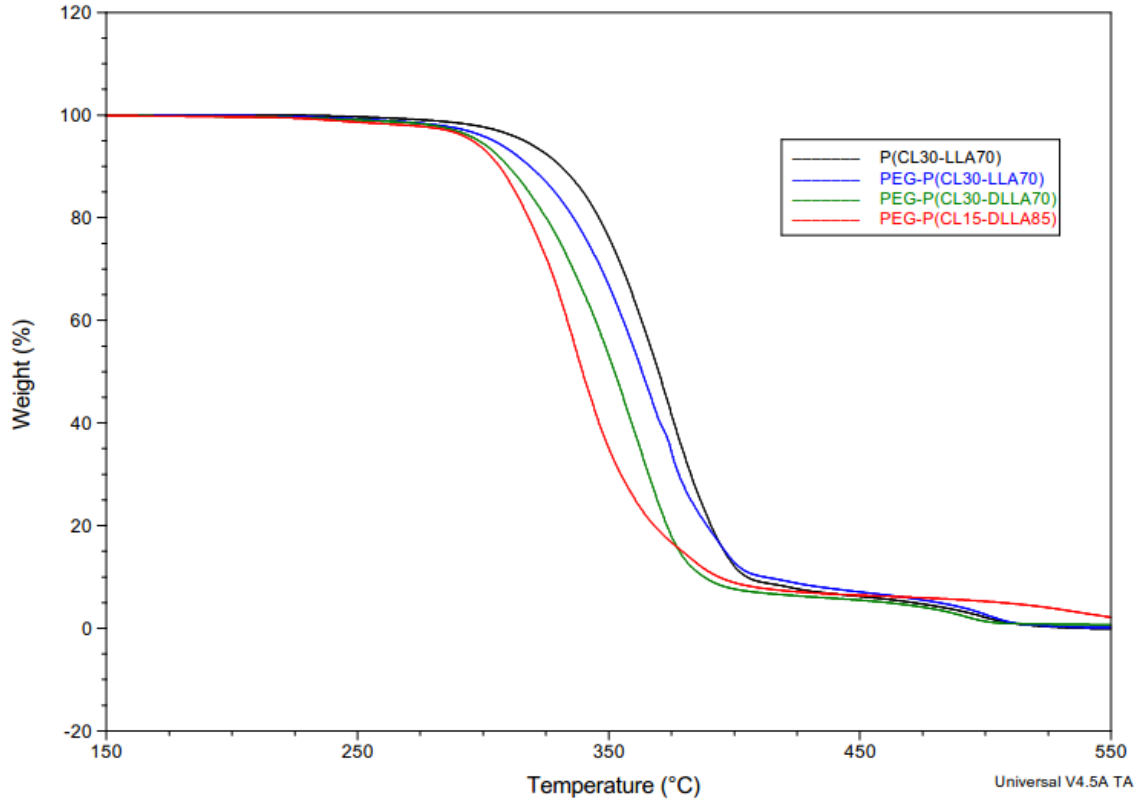



***Figure 33.*** *TGA curves for 8-wt% containing DEX samples. Black line is for P(CL30-LLA70), blue for PEG-P(CL30-LLA70), green for PEG-P(CL30-DLLA70) and red for PEG-P(CL15-DLLA85).*

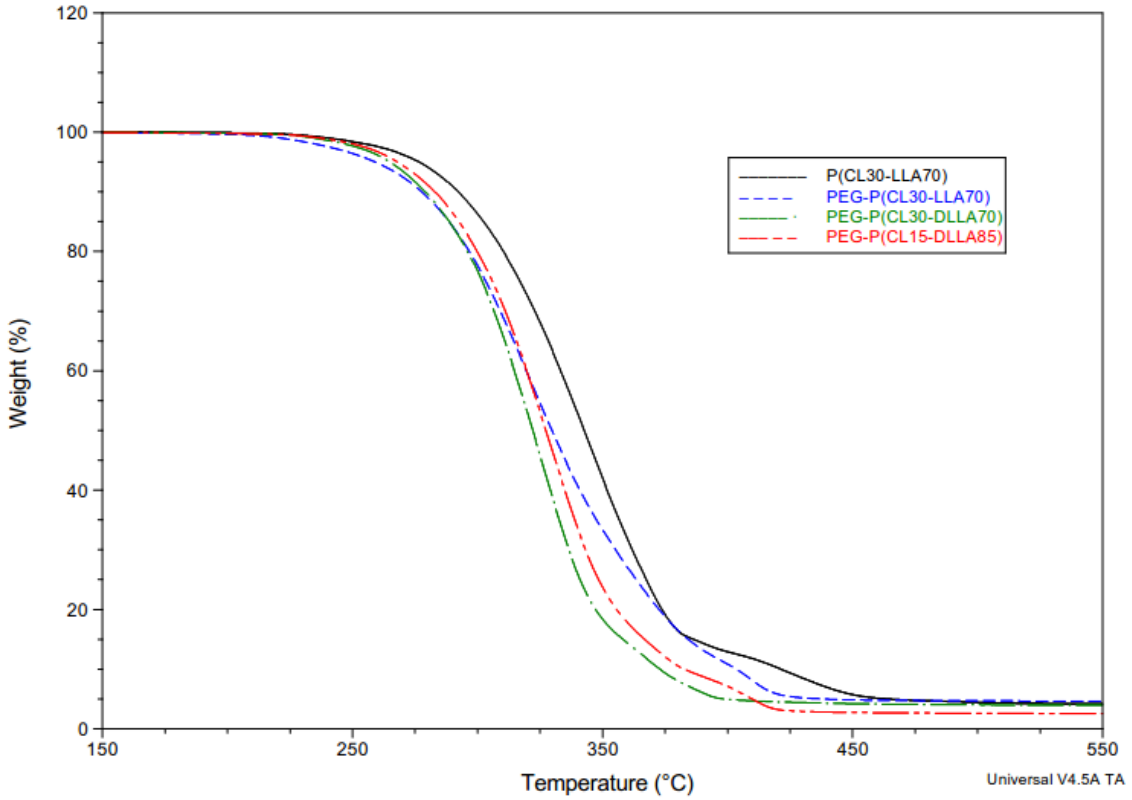



***Figure 34**. TGA curves for 8-wt% containing AAs samples. Black line is for P(CL30-LLA70), blue for PEG-P(CL30-LLA70), green for PEG-P(CL30-DLLA70) and red for PEG-P(CL15-DLLA85).*

As it can be seen from figures, mass starts to decrease earlier from AAs samples than from DEX samples. AAs curves does not reach 0-wt% at end of measurement. It was noticed that there were some residues left in sample container.

## 5.9 microCT

2D images of each kind of samples are presented in *Table 9* and *Table 10*. In ascorbic acid containing samples, it is possible to see white areas which cannot be found from dexamethasone containing samples. These are probably drug that is in dispersed form. It was already known that the drug did not blend very well with used polymers. There are also more white areas in samples containing higher amount of drug. Samples looks very porous except PEG-P(CL30-LLA70) samples.

***Table 9.*** *2D µCT-images of porous samples containing ascorbic acid salt. Length of scale bar is 1mm and it is found from left bottom corner. Unfortunately those are not shown very well in small images.*

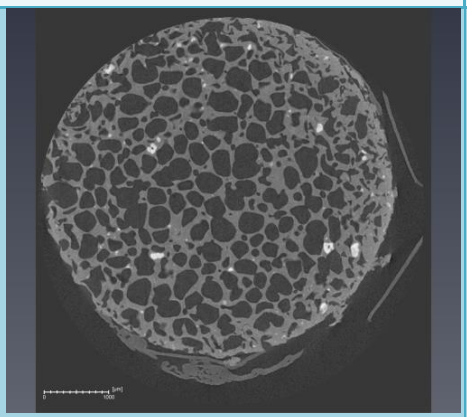
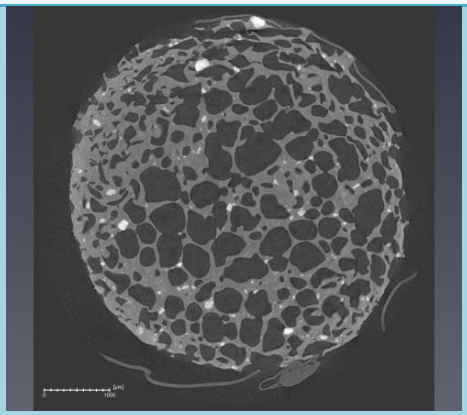
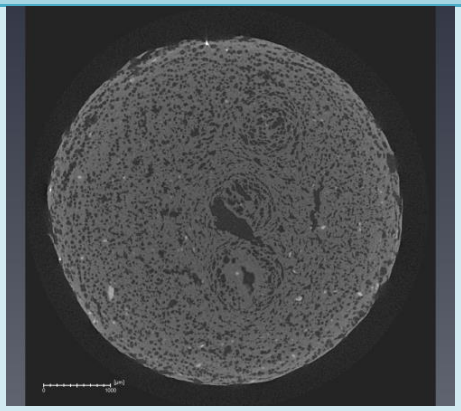
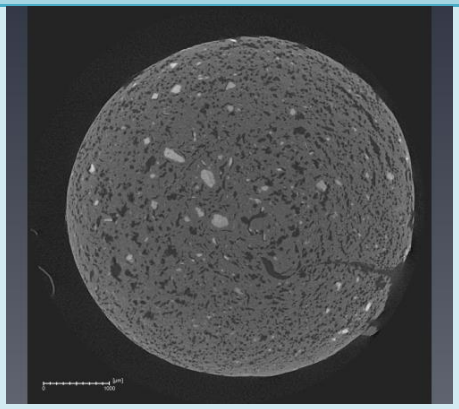
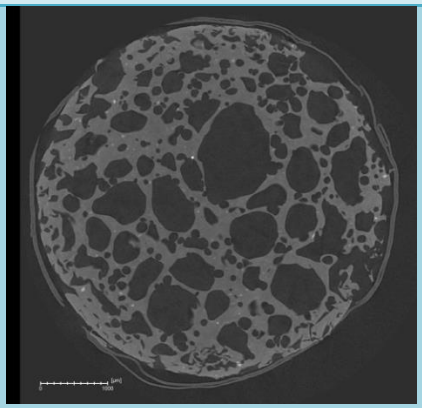
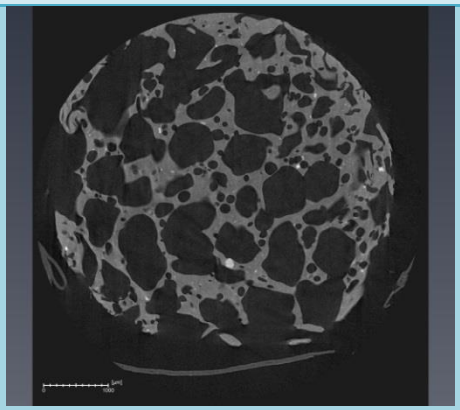
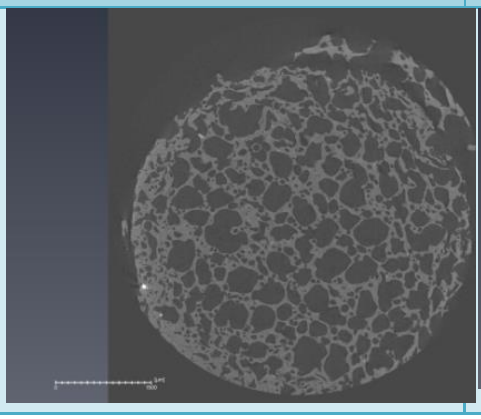
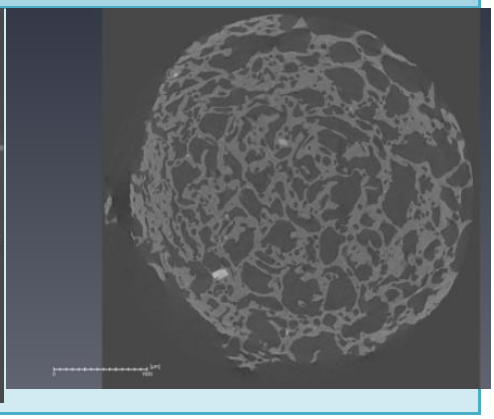
| **Ascorbic acid salt** | **4-wt%** | **8-wt%** |
|---|---|---|
| **P(CL30-LLA70)** |  |  |
| **PEG-P(CL30-LLA70)** |  |  |
| **PEG-P(CL30-DLLA70)** |  |  |
| **PEG-P(CL15-DLLA85)** |  |  |

***Table 10.*** *2d µCT-images of porous samples containing dexamethasone. Length of scale bar is 1mm and it is found from left bottom corner. Unfortunately those are not shown very well in small images.*

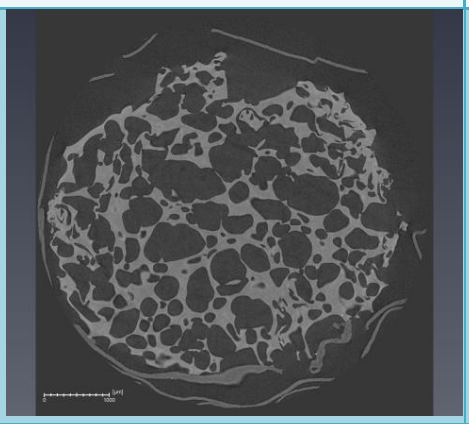
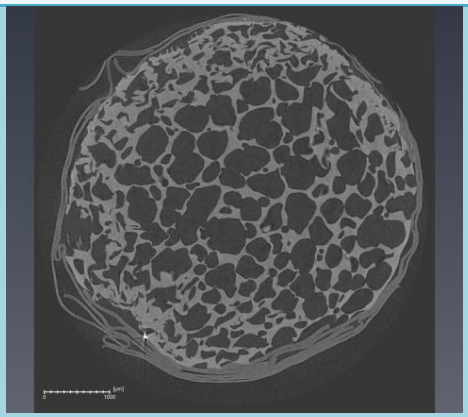
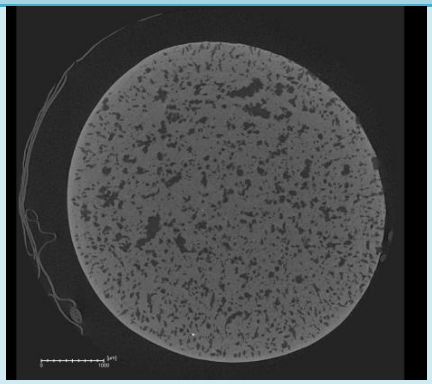
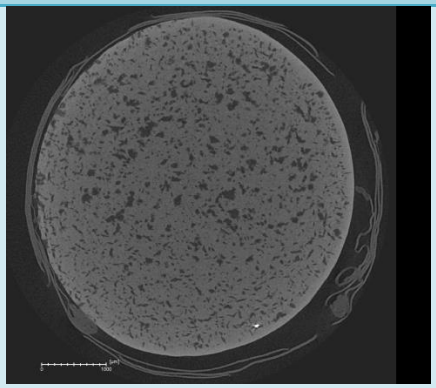
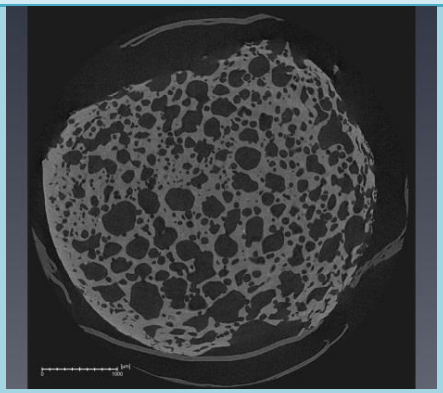
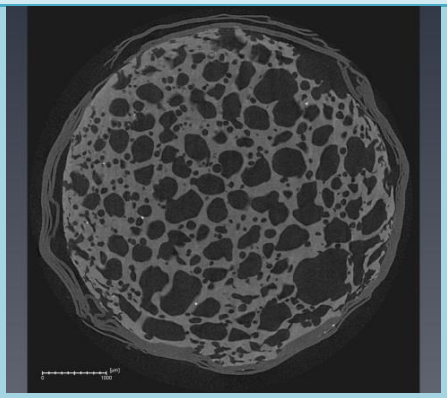
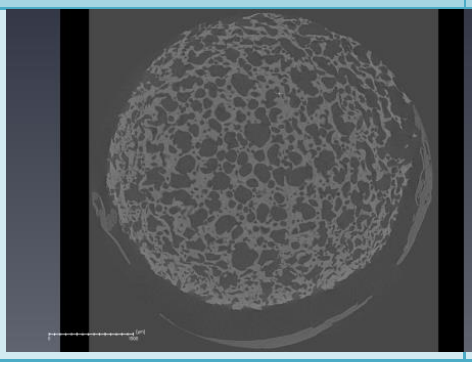
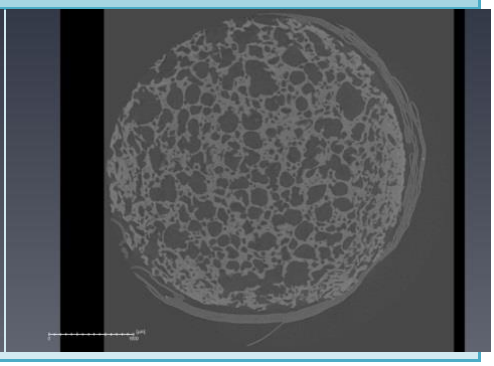
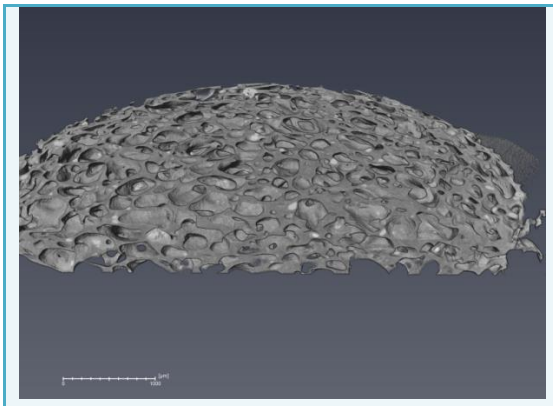
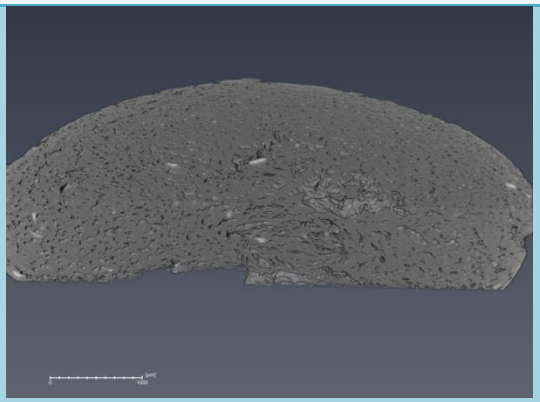
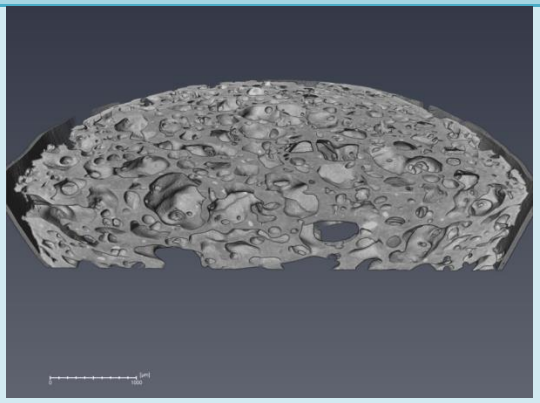
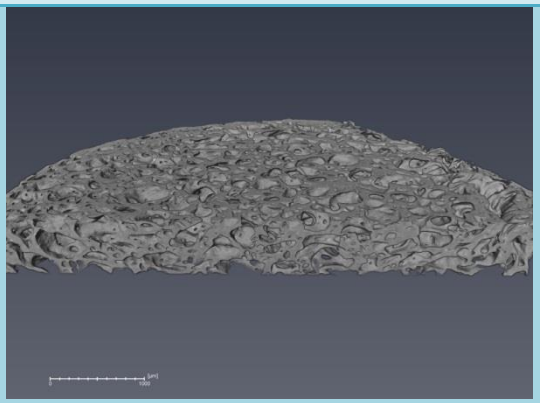
| **Dexame-thasone** | **4-wt%** | **8-wt%** |
|---|---|---|
| **P(CL30-LLA70)** |  |  |
| **PEG-P(CL30-LLA70)** |  |  |
| **PEG-P(CL30-DLLA70)** |  |  |
| **PEG-P(CL15-DLLA85)** |  |  |

***Table 11.*** *3D µCT-images of different porous materials having 4-wt% ascorbic acid salt. Length of scale bar is 1mm and it is found from left bottom corner. Unfortunately those are not shown very well in small images.*

| | |
|---|---|
| **P(CL30-LLA70)** |  |
| **PEG-P(CL30-LLA70)** |  |
| **PEG-P(CL30-DLLA70)** |  |
| **PEG-P(CL15-DLLA85)** |  |

Pores became more visible in 3D images (*Table 11*). Images shown are from 4-wt% ascorbic acid salt containing samples. It looks like PEG-P(CL30-DLLA70) has largest pores and it would have also biggest porosity. Pores may have good interconnectivity. On the contrary, in PEG-P(CL30-LLA70) samples very small pores are seen. It was also possible to do some quantitative analysis. Some results are summarized in *Table 12*.

***Table 12.*** *Analysis done based on CT-images. A means ascorbic acid salt, D means dexamethasone and numbers 4 and 8 theoretical drug contents.*

| Name | Porosity (%) | Void thick-ness mean (µm) | Void thickness std (µm) | Area/Volume |
|---|---|---|---|---|
| **P(CL30-LLA70)** | | | | |
| A4 | 62 | 153 | 62 | 0.0481 |
| A8 | 60 | 171 | 73 | 0.0373 |
| D4 | 65 | 219 | 110 | 0.0391 |
| D8 | 66 | 204 | 90 | 0.0423 |
| **PEG-P(CL30-LLA70)** | | | | |
| A4 | 31 | 58 | 57 | 0.0511 |
| A8 | 21 | 31 | 16 | 0.0402 |
| D4 | 23 | 53 | 56 | 0.0353 |
| D8 | 19 | 43 | 43 | 0.0335 |
| **PEG-P(CL30-DLLA70)** | | | | |
| A4 | 61 | 245 | 147 | 0.0324 |
| A8 | 72 | 313 | 166 | 0.0378 |
| D4 | 57 | 166 | 87 | 0.0402 |
| D8 | 57 | 176 | 99 | 0.0369 |
| **PEG-P(CL15-DLLA85)** | | | | |
| A4 | 64 | 151 | 73 | 0.0557 |
| A8 | 60 | 127 | 67 | 0.0535 |
| D4 | 61 | 114 | 61 | 0.0603 |
| D8 | 61 | 118 | 58 | 0.0586 |

General porosity, pore size with standard deviation and area/volume were analyzed for each sample. Porosities were around 60% in general except for PEG-P(CL30-LLA70) it varied between 19-31%. Mean pore size varied more. For PEG-P(CL30-DLLA70) had largest (166-313µm) pores which was already possible to see from CT-images. PEG-P(CL30-LLA70) had smallest pores (31-58µm).

Low porosity of PEG-P(CL30-LLA70) was somewhat surprising because it had relatively low i.v. and molecular weight compared to other polymers used. However crystallinity is suggested to have effect how well pores are formed due to morphological

effects to $CO_2$ solubility to polymer (Davies et al. 2008). Sheridan et al. (2000) Prepared porous PLGA scaffolds using high pressure $CO_2$. In their work, samples having high molecular weights did not have high porosity of same material with different molecular weights. It was suggested that longer polymer chains had more resistant against expansion than shorter ones had. In their work, the amorphous PLGA polymers foamed better than homopolymers of each monomer. It was suggested that $CO_2$ had better dissolution into amorphous regions. (Sheridan et al. 2000) PEG-P(CL30-LLA70) was only polymer that had some crystallinity when samples were processed and can possibly explain why other polymers gained better porosity.

Area/volume ratio varied approximately 0.0324 to 0.0603. No clear trends are seen at this point.

## 5.10 Effect of drug properties

It seems that properties of drugs had the most dominant effect on release profiles with used polymers. Used drugs were very different in nature and it was seen in release behavior. Ascorbic acid salt is very well-soluble in water whereas dexamethasone has very low solubility. On contrary, AAs is not soluble in many other solvents. It was noticed already in blending that AAs did not blend well in the polymers used. Also standard deviations in measurements of initial drug contents and actual drug release measurements were relatively high which indicated that drug was not homogenously dissolved in polymer. Also μCT-images showed that AAs was dispersed in polymer since white areas were clearly seen in pictures. Similar white areas were not seen in dexamethasone containing samples. Standard deviations from dexamethasone samples were much smaller in initial drug content measurements and in actual drug release measurements.

Release patterns were very different. AAs released either very fast or release was almost negligible until samples degraded. AAs probably had very weak interaction with matrix polymers.

Similar kind of conclusions was done by Dorj et al. (2014) by preparing porous PLLA scaffold for drug release with porosity of approximately 70%. Hydrophilic anionic Ampicillin released really fast. Approximately 85% was released during first week. It was suggested that drug and polymers had weak chemical interaction. However another drug used, Cytochome C came out in very different way. After burst release, cytocine C was released in sustained way up to 28 days. Release remained in steady state after burst. Cyticine C has positive charge and is expected have interactions with polymer. For both drugs, nonporous samples were also prepared. Since drugs were loaded into scaffolds by soaking samples in drug loaded solution, loading into nonporous samples were relatively poor and release was not steady. (Dorj et al. 2014)

It was also suggested that differences in release profiles from same material may be due to differences in drugs functional groups that can react differently with hydrogen bonds of polyester (Jelonek et al. 2013). Our drugs has quite different structures but size

does not differ much. Dexamethasone has molecular weight of approximately 392 g/mol and AAs 322 g/mol.

Lee et al. (2009) studied well water soluble α-lipoic acid release from PEG-P(CL60-DLLA40). Drug was blended homogenously in copolymer including PEG, but distributed on the surface of polymer samples without PEG. Used PEG were 350 g/mol. After 24 hours, 90 % of drug was released from samples without PEG and 50 % from samples with PEG. In our study, AAs were poorly soluble to used materials and when PEG was incorporated to structure, release became faster. Here, it can be concluded that even solubility of drug plays important role and can be dominant factor affecting the release of drug.

# 6 CONCLUSIONS

In vitro drug release test series was done to four different copolymers. Lactide and caprolactone were synthesized in presence of ethylene glycol to produce block structure where PEG is in middle of chain. Commercial P(CL30-LLA70) was used to compare results of experimental materials. Characterization like differential scanning calorimetry, thermogravimetric analysis, capillaryviscometric analysis and size-exclusion chromatography was used materials to understanding better behavior of drug-material relationship. Samples were prepared using two different drugs, two drug contents (4-wt% and 8-wt% in feed) and porous and nonporous samples.

Main interest was in the drug release. Drug release was monitored using UV/VIS-spectrophotometer. Many factors affecting to release behavior were found. In general PEG incorporation into backbone increased release rates for all materials and for AAs samples, it changed release profiles too. It was known that PEG increases hydrophilicity, which makes water absorption to polymer easier and affects the degradation kinetics by increasing the release rate.

By changing type of lactide, from L-lactide to DL-lactide, release from solid samples became very minimal, for most samples, negligible. These amorphous materials changed release rates when they were processed with $sCO_2$. Dexamethasone was released before samples were too degraded for measuring and AAs was released in very fast way.

When DL-content was increased, the release profiles remained similar, but release rates increase slightly with both drugs. Content of drugs did not affect much to release profile in general, but it was possible to tailor release rates.

Most of all, properties of drugs had a great effect on the release. AAs had relatively weak interaction with matrix polymers, while dexamethasone did not show any signs of being in dispersed form. With dexamethasone, sustained nearly zero-order kinetics was possible to achieve for over 120 days for porous semi-crystalline polymers used. Processing samples with super critical $CO_2$ increased release rates of all samples.

Suggestions to future work would be doing degradation test series that would help understanding the release behavior of used materials better. Even though, there are literature found about degradation of similar materials, it was noticed that drug can effect also to whole implant and degradation. More than one week delay at beginning of drug release was seen in commercial AAs samples with different drug contents. Also release test series were finished one week earlier to some samples due to faster degradation. Changes of materials properties, especially changes in structure would be interesting to monitor and compare how well these changes correlate with changes in release profiles.

Additionally, more characterization to understand better the complex relationships of factors affecting the release is necessary.

These materials clearly have great potential in drug release applications. Especially dexamethasone could potentially be used in applications that needs long term drug release. This was a pilot study and there was not made effort to consider whether results were therapeutic doses of not. Also samples were chosen not to be sterilized before test series. Testing sterilized samples would be highly recommended since it is known to affect material properties.

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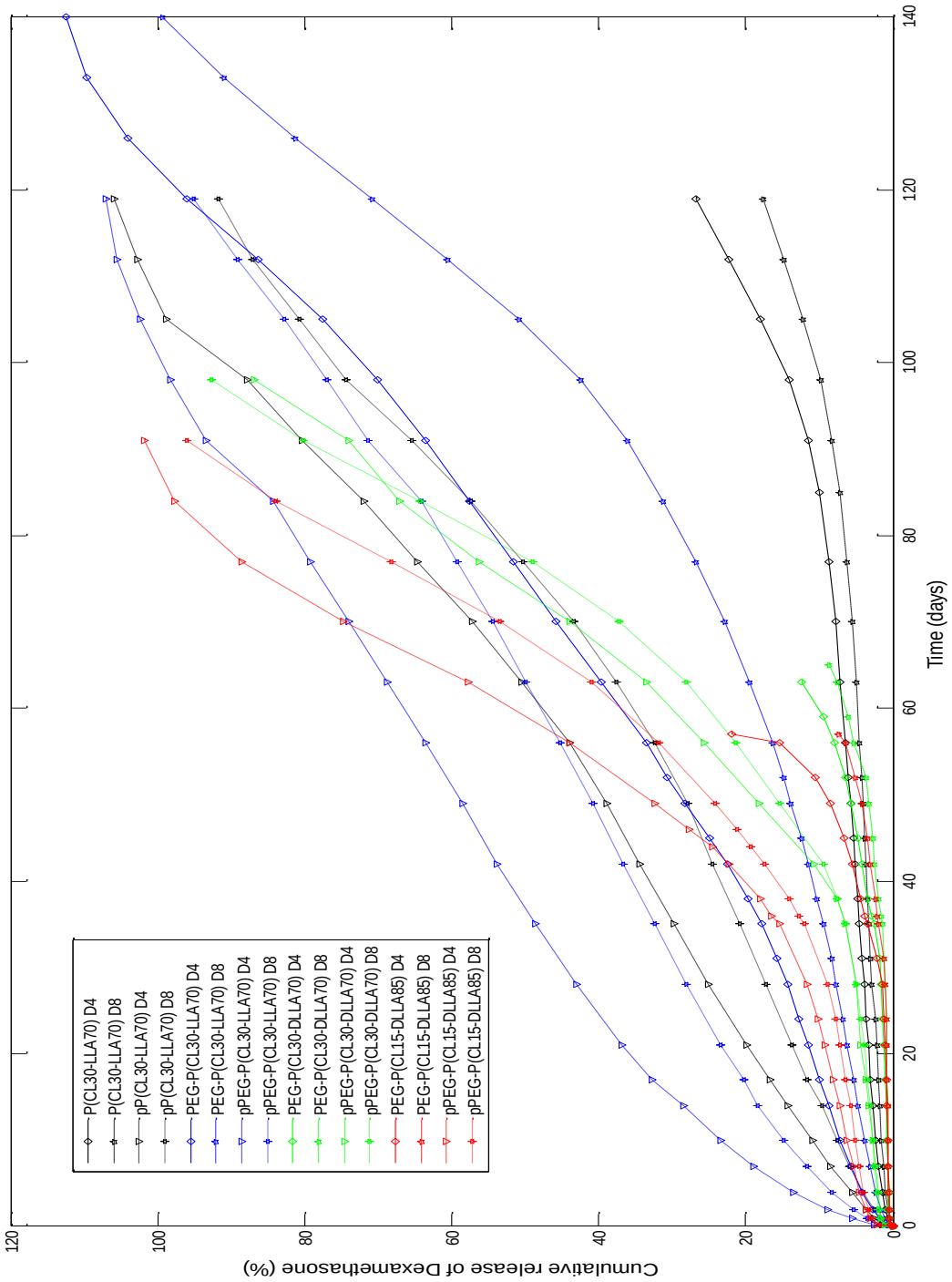
# APPENDIX A: RELEASE OF DEXAMETHASONE



# APPENDIX B: RELEASE OF ASCORBIC ACID SALT